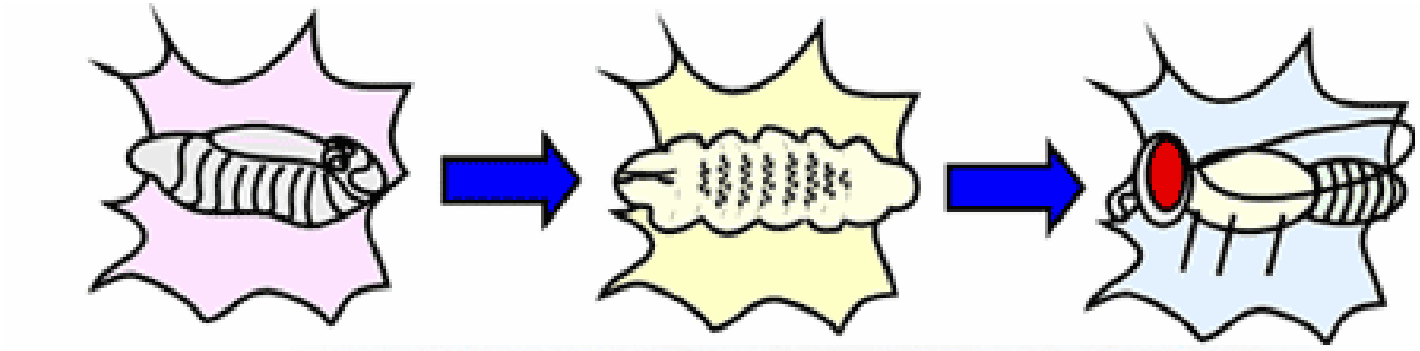
Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 24

Gene Regulation during Drosophila Development



An organism arises from a fertilized egg as a result of three key processes:

Cell division, Cell differentiation and Morphogenesis

Following fertilization, as the egg starts dividing and development proceeds, cells which are initially undifferentiated (Embryonic stem cells) ultimately differentiate into specific cell types.

Differential expression of genes in the embryonal cells plays a key role in their differentiation into diverse cell types, tissues and organs during development.

Regulation of gene expression during development is directed by:

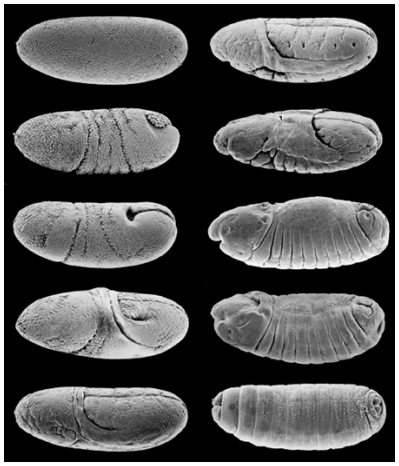
Maternal molecules in the fertilized egg's cytoplasm
Extracellular signals and cell-cell interactions

In animals, development & differentiation begin in early embryo:

First, the basic body plan is established

Next, Major axes are established (Dorsal, ventral, anterior, posterior)

Gene regulation during embryonic development is studied in a number of model organisms such as *Drosophila melanogaster, Cenorhabditis elegans, zebra fish, mouse* etc.



Drosophila melanogaster

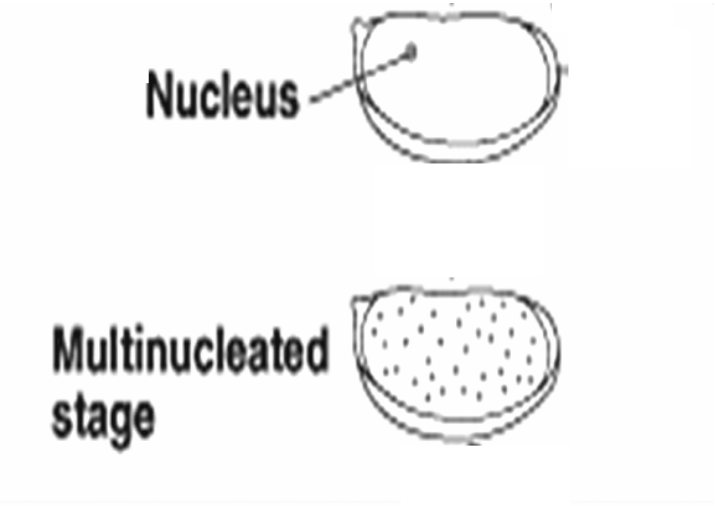
Bilateral symmetry
Segmented body that is
divided into
Head, Thorax and Abdomen

Drosophila melaogaster
Early embryonic development

After fertilization, the diploid, zygotic nucleus undergoes 10 rapid cleavages.

At this stage, the embryo is called a syncitium because it is a single cell with multiple nuclei.

After 8 cleavages, the resulting 256 nuclei begin to migrate to the outer edge of the cell.



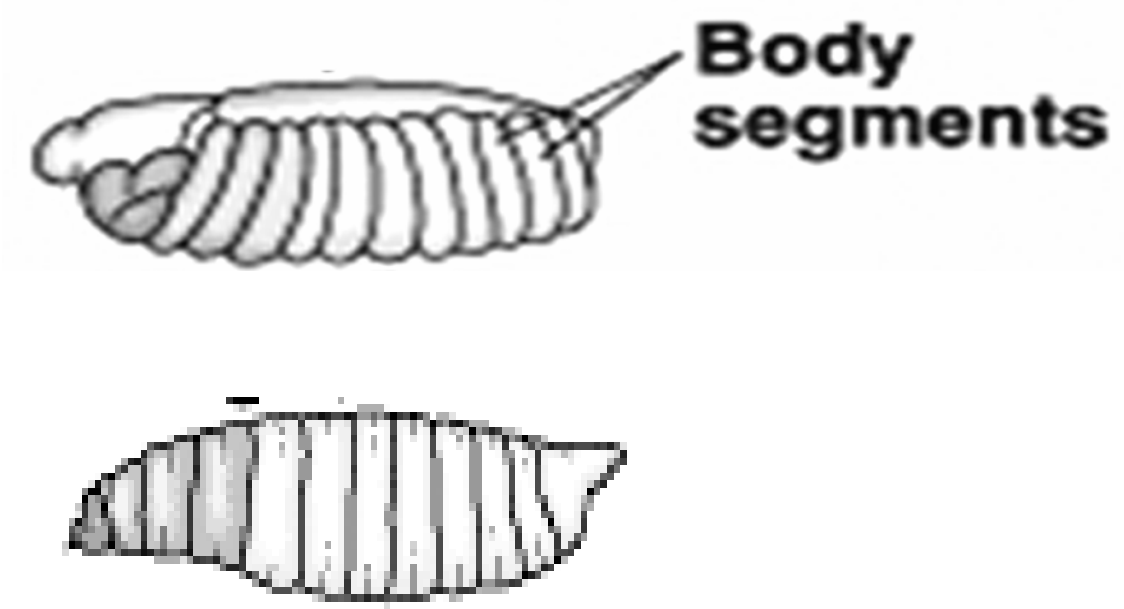
About 90 minutes after fertilization, most nuclei have reached the periphery. These nuclei undergo 3 more cleavages.

Plasma membranes finally partition ~6,000 nuclei into separate cells at the thirteenth division. By this time the basic body plan (Body axes, segments, boundaries) is already determined.

When the nuclei reach the periphery, they are totipotent.

Just after cellularization, the nuclei lose their totipotency and are destined to differentiate into specific tissues in the adult fruit fly. The location each nucleus determines its fate.

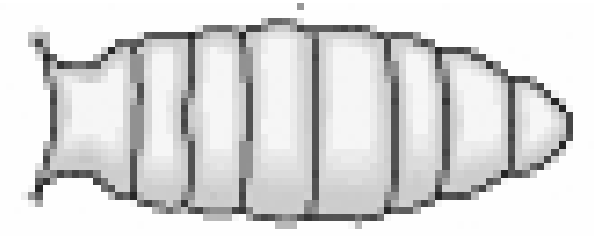
Soon the cells undergo differentiation and specific organs are formed. A wormlike larva hatches eats, grows, & molts

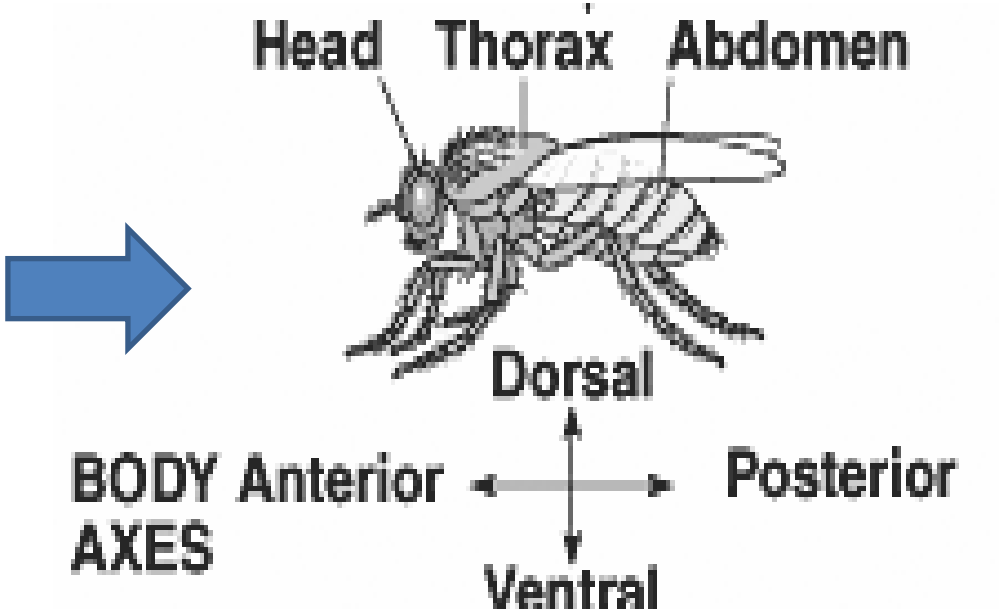


Larva eventually forms a pupa

Metamorphosis occurs

Adult fly emerges with each segment that is anatomically distinct





What are the molecular events that are involved early embryonic development?

Gradients of maternal molecules in the early embryo control axis formation

Cytoplasmic determinants already present in unfertilized egg encoded by mother's *maternal effect genes* *a.k.a.,* "*Egg-polarity genes*" encode proteins or mRNAs that are placed into the egg while still in the mother's ovary

One group of maternal effect genes establishes the anterior-posterior axis of the embryo

Another set of maternal effect genes establishes the dorsal-ventral axis

Female flies possessing mutations in *maternal effect genes* appear phenotypically normal, but produce offspring with mutant phenotypes

Asymetric distribution of mRNAs during cell division such that the daughter cells inherit different amounts of these mRNAs.

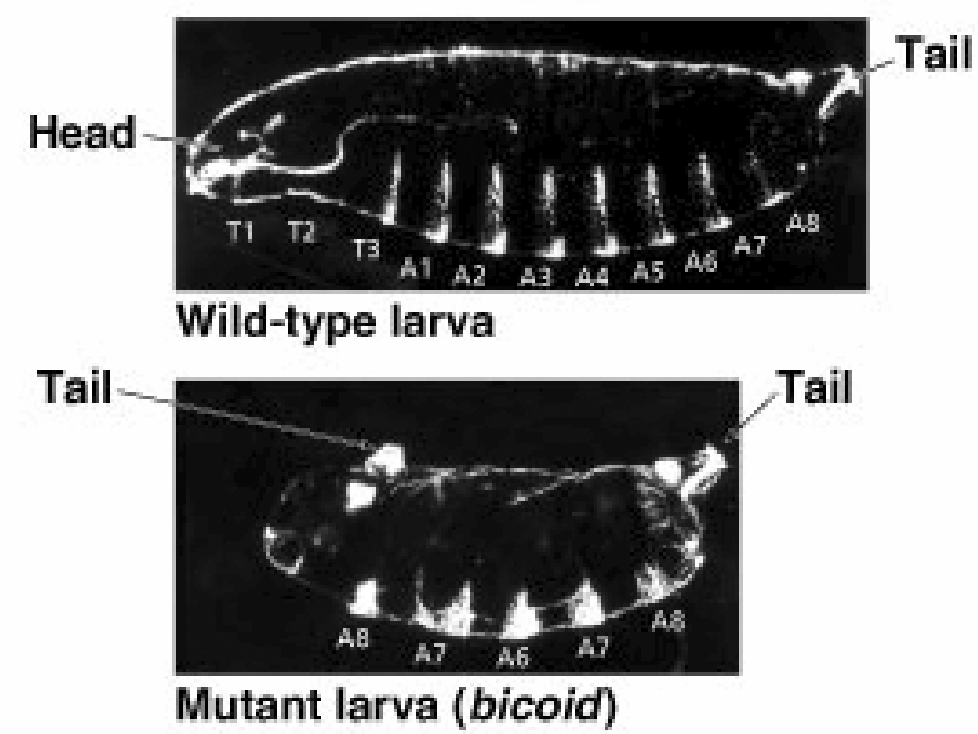
Distribution of mRNAs in unfertilized egg

Redistribution of mRNAs to specific regions after fertilization

These mRNAs often encode:
RNA-binding proteins
cell signalling molecules
transcriptional activators and repressors

For example……Bicoid

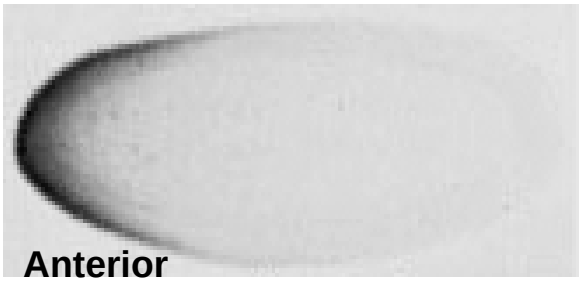
- ***Bicoid*** is an egg-polarity gene
- Mothers defective in *bicoid* produce embryos lacking the front half of their body
  - Duplicate posterior structure at both ends



- ***Bicoid*** gene product is concentrated at anterior end of fly embryo
  - Gradient of gene product
  - Essential for setting up anterior end of fly

- Gradients of other proteins determine the posterior end and the dorsal-ventral axis

Bicoid mRNA in unfertilized egg



Bicoid protein in early embryo

What is Bicoid?

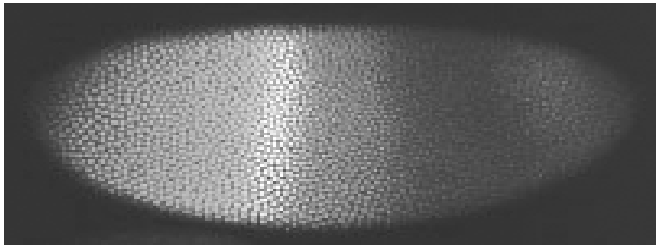
The *bicoid* protein as well as the products of other egg-polarity genes are *transcription factors* which regulate the expression of some of the embryonic genes

Once the embryo's major axes are formed, the formation of segments is regulated by segmentation genes

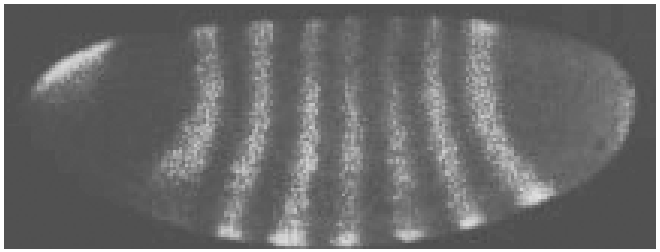
Activation of three sets of segmentation genes defines the body plan: are activated sequentially

- Gap genes
- Pair-rule genes
- Segment polarity genes

Gap genes are responsible for basic subdivisions along the embryo's anterior-posterior axis and mutations in these genes cause "gaps" in the animal's segmentation



Pair-rule genes define pattern in terms of pairs of segments and mutations in these genes result in embryos having half the normal number of segments



Segment polarity genes set the anterior-posterior axis of each segment and mutations in these genes produce segments where part of the segment mirrors another part of the same segment.

The products of many of the segmentation genes are transcription factors which activate the next set of genes.

Summary

Products of the egg-polarity genes regulate the regional expression of the gap genes

Gap genes control the localized expression of the pair-rule genes

Pair rule genes activate specific segment polarity genes in different parts of each segment

Segment polarity genes activate homeotic genes

Hierarchy of Gene Activation

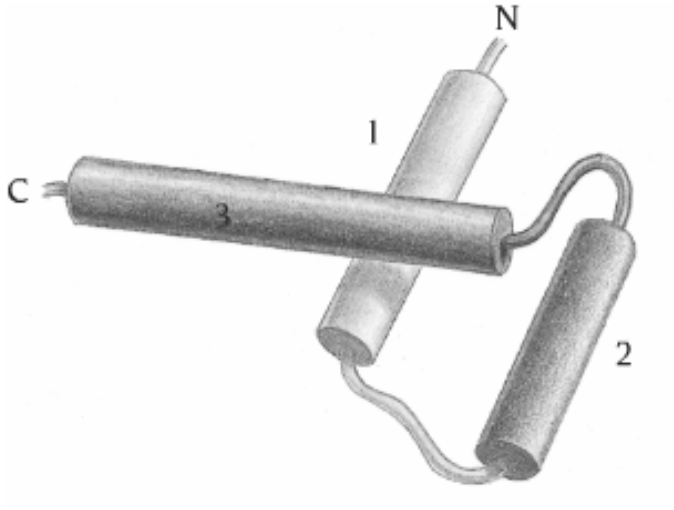
- Maternal genes
- Segmentation genes of embryo
  - Gap genes
  - Pair-rule genes
  - Segment polarity genes
- Homeotic genes of the embryo
- Other genes of the embryo

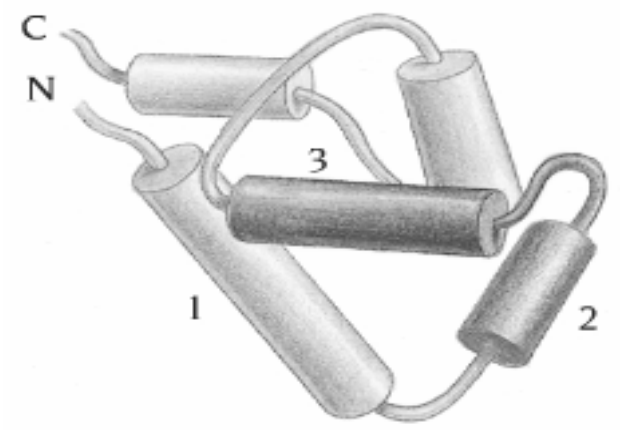
HOMEOTIC GENES are master regulatory genes which specify the types of appendages and other structures that each segment will form. Mutations in homeotic genes produce flies with structures in incorrect places

Homeotic gene products are transcription factors which control the expression of genes responsible for specific anatomical structures

For ex., where Antennae should form, where legs should appear etc.

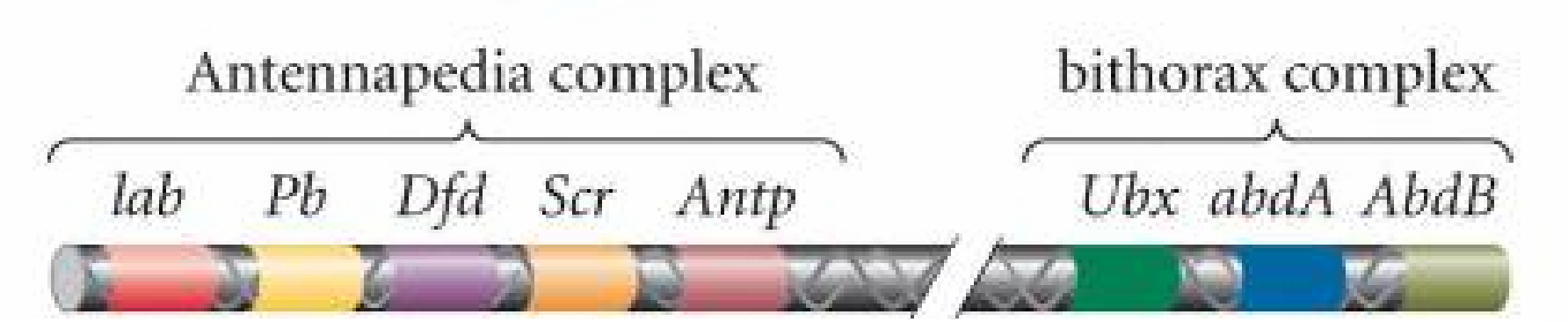
Homeotic genes of *Drosophila* possess a 180-nucleotide sequence known as the **homeobox** which encodes a 60-amino-acid region called **homeodomain** that functions as a DNA binding domain.





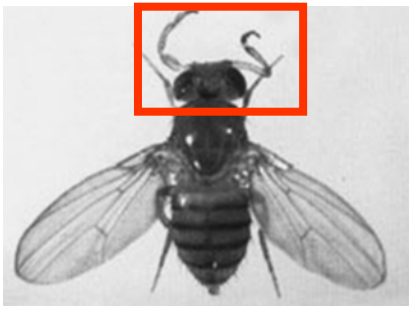
The homeodomain consists of 3 helices in which helices 2 and 3 form helix-turn-helix motif similar to those present in in prokaryotic DNA binding proteins such as the λ repressor.

Homeotic Genes of *Drosophila*



HEAD THORAX ABDOMEN

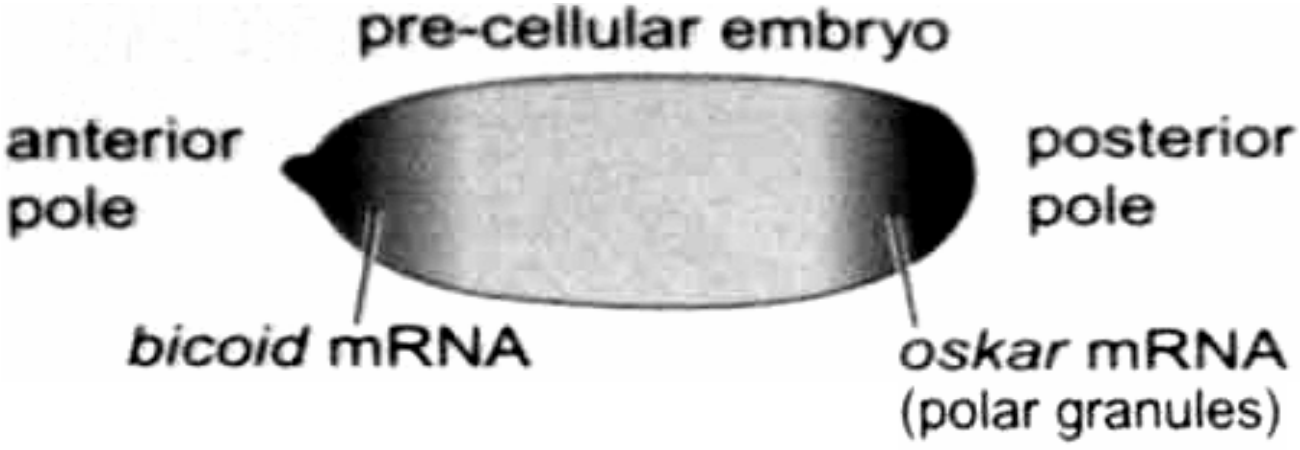
Deletion of Ubx gene transforms T3 into T2



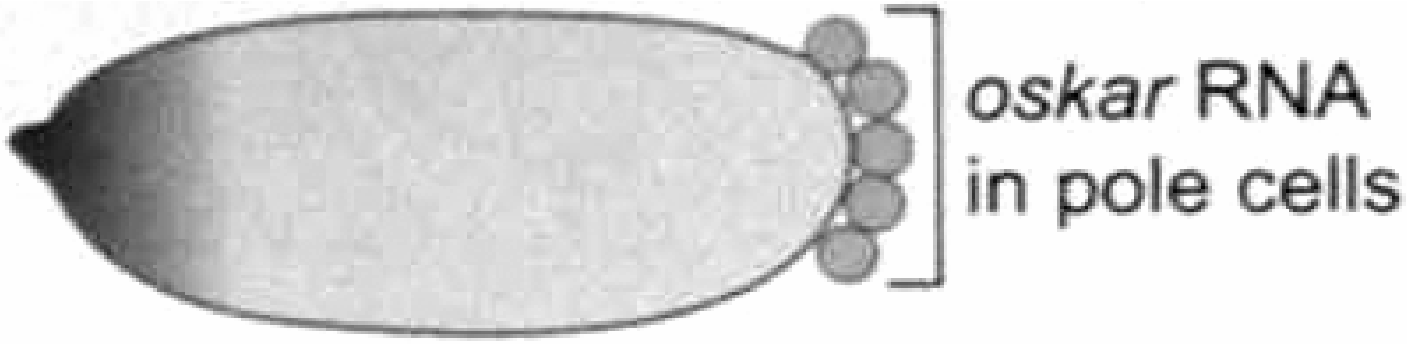
Legs sprouting from head in place of antennae.

Segmentation is initiated by localized RNAs at the anterior and posterior poles of the unfertilized egg.

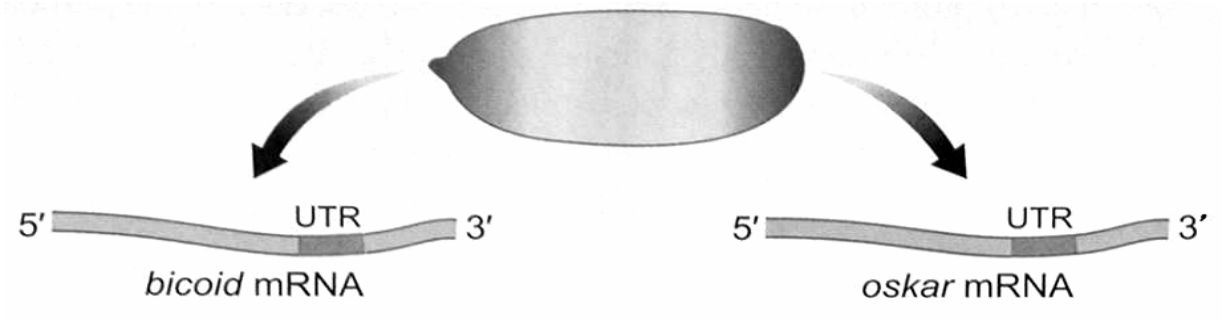
The unfertilized egg contains two localized mRNAs, *bicoid* in anterior regions and *osker* in posterior regions.



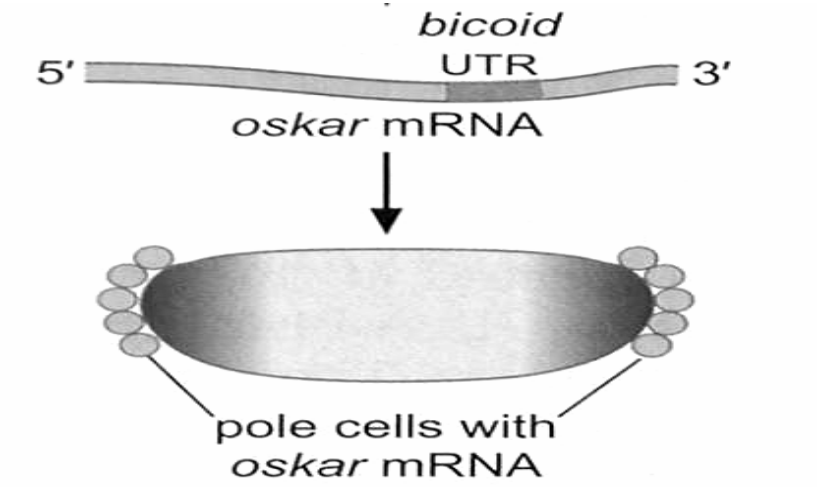
The *osker* mRNA encodes an RNA-binding protein that regulates the assembly of **polar granule**, which control the development of tissues arising from posterior regions of the early embryo.



The *bicoid* UTR causes it to be localized to the anterior pole while the *oskar* UTR causes localization in the posterior region.

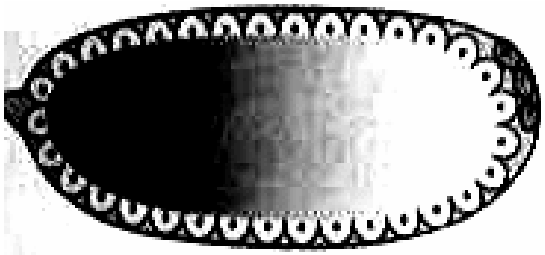


A modified *oskar* mRNA that contains the *bicoid* UTR is localized to the anterior pole. This mislocalization of *oskar* causes the formation of pole cells in anterior regions.

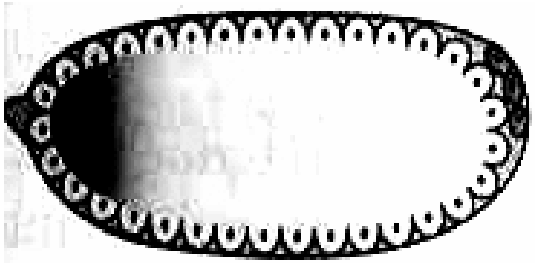


The bicoid gradient regulates the expression of segmentation genes in a concentration-dependent fashion.

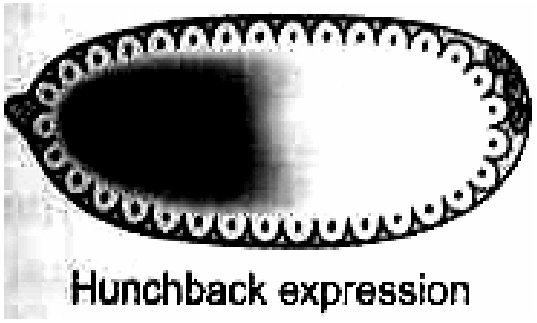
There are high levels of the Bicoid protein in anterior regions, intermediate levels in central regions, and low levels in posterior regions.



Orthodenticle is activated only by high levels of the Bicoid gradient in the head



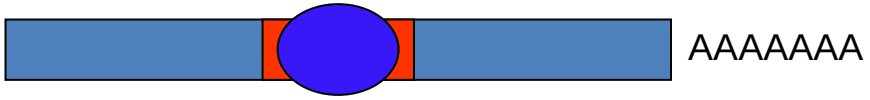
hunchback is activated by both high and intermediate levels in the head and thorax.



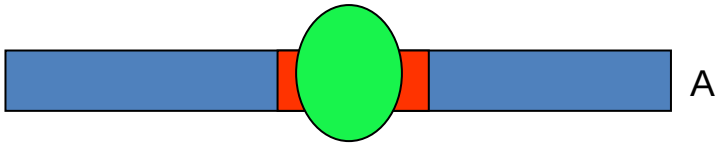
Hunchback expression is also regulated at the level of translation

The translation is blocked in posterior regions by RNA-binding protein called **Nanos**. After the *Nanos* mRNA is translated, the protein diffuses from posterior regions to form a gradient. The translation of the maternal *hunchback* mRNA is arrested by the Nanos protein. The Nanos gradient thereby leads to the formation of a reciprocal Hunchback gradient in anterior regions.

Control of *hunchback* mRNA Translation by Nanos Protein



NanosResponse element



NanosResponse element

polyadenylation

Deadenylation

Translation of
Hunchback mRNA

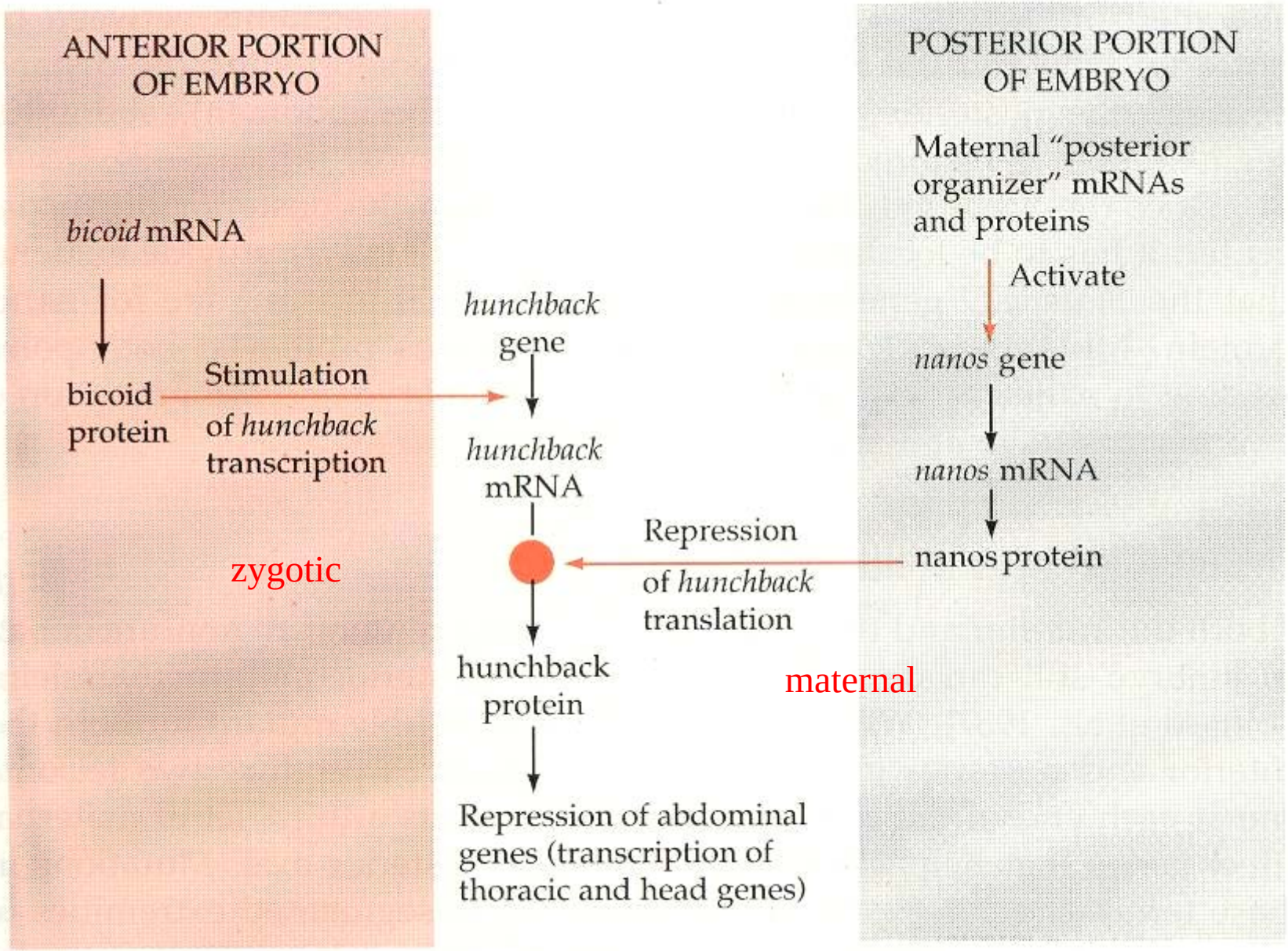
No Translation of
Hunchback mRNA

Hunchback protein

No Hunchback protein

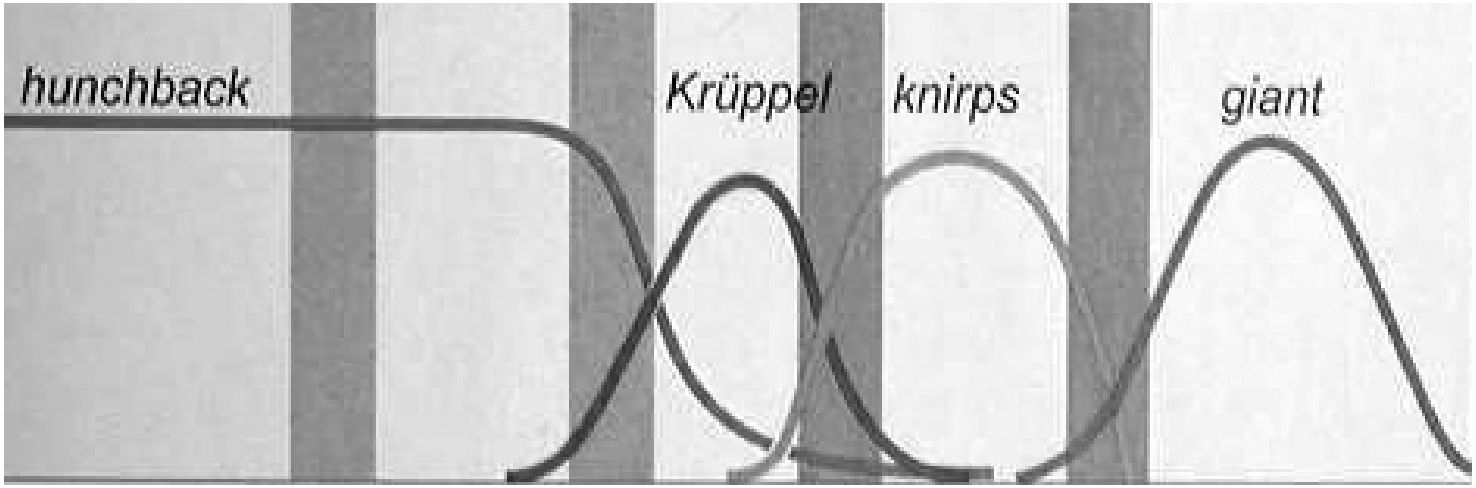
Anterior structures are formed

Abdominal structures are formed


ANTERIOR PORTION OF EMBRYO
bicoid mRNA
bicoid protein
Stimulation of hunchback transcription
zygotic
hunchback gene
hunchback mRNA
hunchback protein
Repression of abdominal genes (transcription of thoracic and head genes)
POSTERIOR PORTION OF EMBRYO
Maternal "posterior organizer" mRNAs and proteins
Activate
nanos gene
nanos mRNA
nanos protein
Repression of hunchback translation
maternal

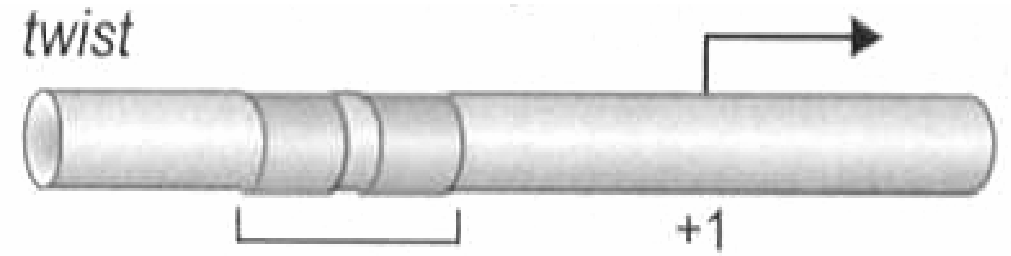
The gradient of hunchcack repressor estiblishes different limits of gap gene expresion.

High levels of Hunchback are required for the repression of *Krüppel*, whereas intermediate and low levels repress the expression of *knirps* and *gaint*, respectively.



Let us now examine how levels of a transcription factor known as the Dorsal can regulate the expression of three different target genes (twist, rhomboid and sog)

The highest levels of Dorsal protein activate the expression of the twist gene since the twist 5'regulatory DNA contains two low-affinity Dorsal binding sites.



Two low affinity
Binding sites for
Dorsal.

Occupied at high
Dorsal concentrations

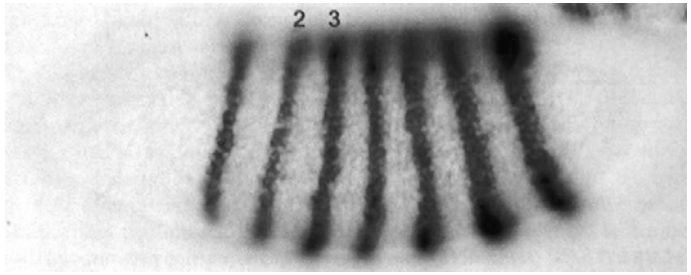
The intermediate levels of the Dorsal protein activate the rhomboid gene.

The lowest levels of the Dorsal protein activate the sog gene.

The *eve* locus contains over 12kb of regulatory DNA. The 5’ regulatory region contains two enhancers. These control the expression of stripes 2,3,and 7. Each enhancer is 500bp in length. The 3’ regulatory region contains three enhancers. These contain the expression of stripes 1,4,and 6.

Each of the 500bp enhancers contains a total of twelve binding sites for the transcriptional activators Bicoid, Hunchback, transcriptional repressors, Krüppel,and Giant proteins.

Complex interactions between these transcriptional activators and repressors and the five enhancers of the eve regulatory regions regulate the expression of the seven eve stripes in the early embryo.



The genes which control the early stages of Drosohila development
Encode transcription factors

At each stage, factors in one area of the egg control the synthesis of other factors that define smaller areas

Maternal genes are expressed during oogenesis and act after fetilization

Three groups of segmentation genes expressed after fertilization control the number and polarity of segments

Finally, the homeotic genes control the identity of the segment.

The bicoid mRNA localized at the anterior end of the egg generates a gradient of protein that extends along the anterior 40% of the egg

The concnetration of bicoid protein determines the types of anterior (head) structures that are formed in each region

The nanos mRNA localized at the posterior end of the egg generates a gradient of nanos protein that extends along the abdominal region.

The function of nanos is to repress genes whose products interfere with posterior development

These mRNAs which determine the anterior and posterior structures are transcribed in the nurse cells and transported through the cytoplasmic bridges into oocytes.

Biocoid mRNA is localized close to the point of entry while oskar and Nanos mRNAs aare transported the length of the oocytes to the posterior end.

Movement of these mRNAs is accomplished by a motor attached to microtubules.

Spatial and temporal expression of genes play a key role in the regulation of early embryonic development of Drosophila

Patterns of Gene Expression During *Drosophila* Mesoderm Development

Eileen E. M. Furlong, Erik C. Andersen, Brian Null, Kevin P. White, Matthew P. Scott

Science, 293, 1629, 2001

GENES VIII by LEWIN

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http://homophila.sdsc.edu/