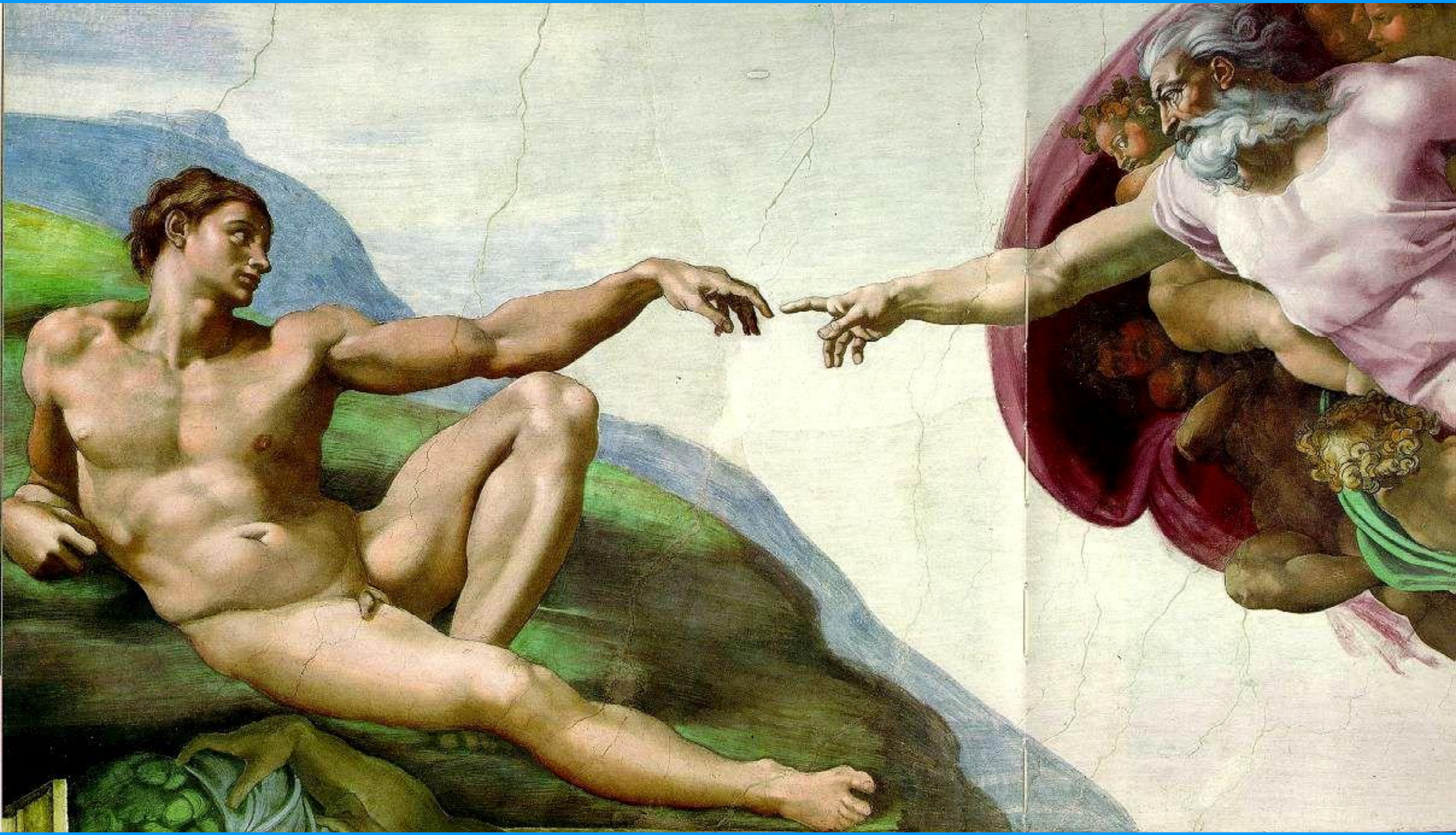
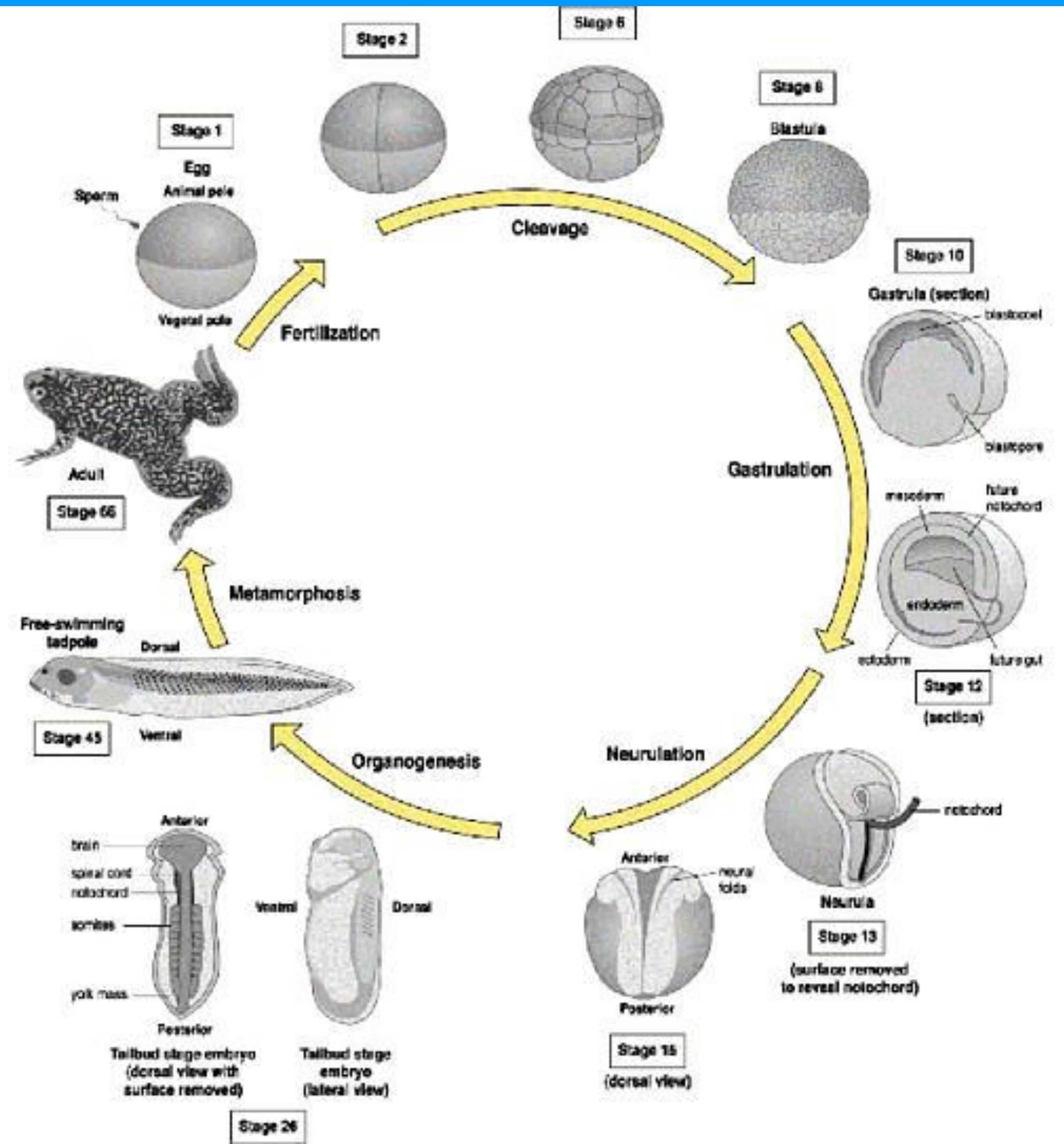


Молекулярная генетика развития

Вельков В В   2013

Gene Regulation during Development

Morphogenesis - How do you get from a spherical egg to say a frog?



Dolly and Bonnie

The nucleus from an differentiated adult cell (from the mammary gland) was able to replace the nucleus of a fertilized egg and produce an adult complete with functional gametes: Dolly had a daughter – Bonnie. SO: during development genes are just turned on and off in a carefully orchestrated fashion. SO, THE DOGMA OF DEV. BIOL. Is differential gene expression controls development

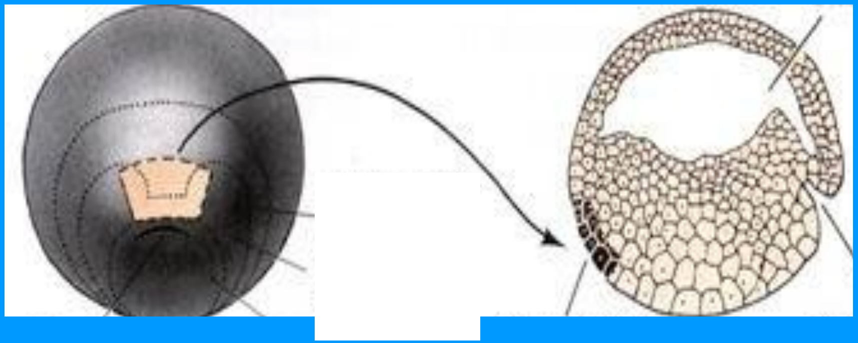
Gilbert, SF (2003) Developmental Biology, 7th ed.

The Spemann Experiment

Spemann & Mangold, *1923*

Spemann Experiment

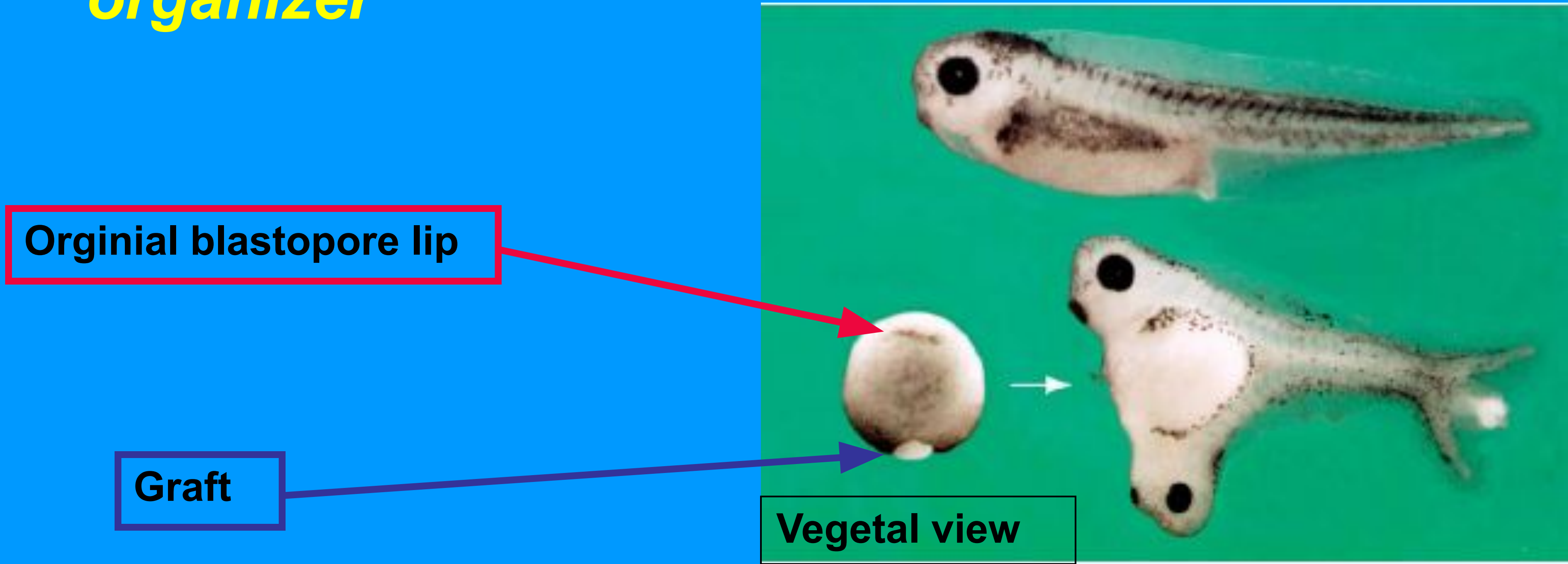
- Outline: Graft a tissue from one embryo into another embryo, and see what happens – **Cut and Paste**



- **Main observation: A graft of a specific tissue (the <u>organizer</u>) to a specific location can induce Siamese twins connected at the belly.**
- Conclusion: **Hilde Mangold and Hans Spemann found that a piece of Prospective dorsal tissue can organize formation of an embryo**

“A piece taken from the upper blastopore lip of a gastrulating amphibian embryo ***exerts an organizing effect on its environment*** ...

Such a piece can therefore be designated as an ***organizer***”



- Spemann won the Nobel prize in 1935
- Hilde Mangold died in 1926…
- “Spemann Organizer” was found
  in all vertebrates, including human

The organizer secretes both **morphogens**
and their inhibitors which diffuse
throughout the embryo

Universal mechanism of animal development

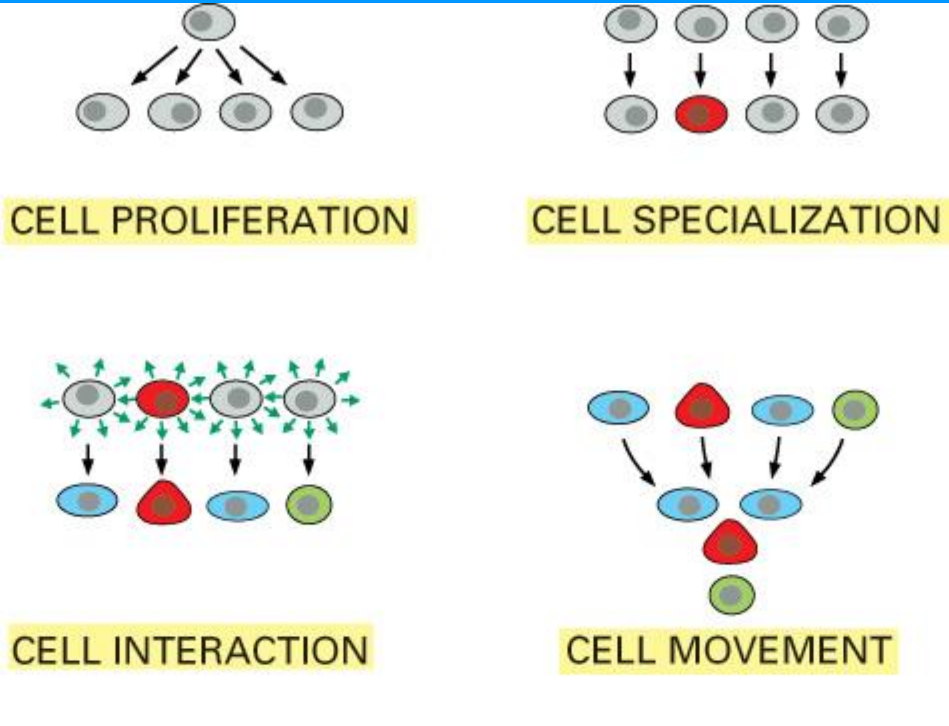


Figure 21–1. Molecular Biology of the Cell, 4th Edition.

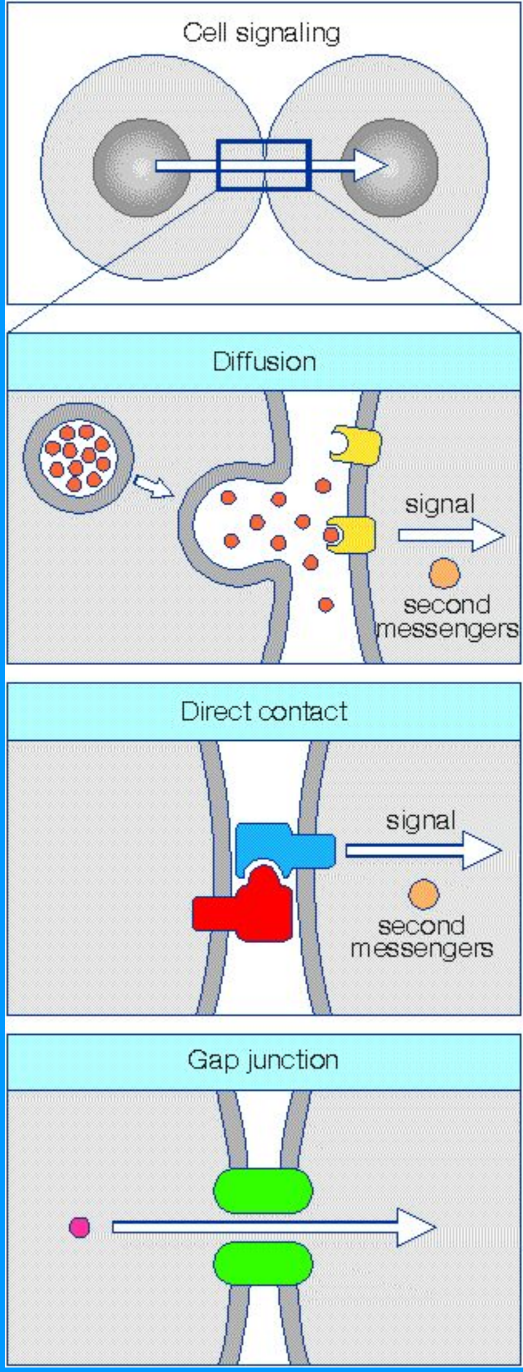
Changes in cell adhesion

Short- long-range diffusible signaling molecule

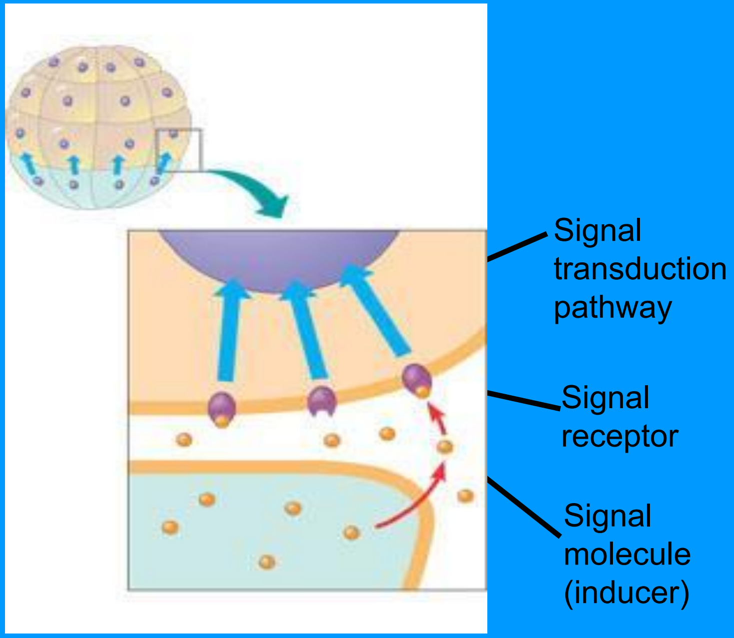
Gene expression controls 4 essential process

Development is progressive

- **Specification of cell fate: determination**
  - **All cells still ‘look the same’**
  - **Can be tested by transplantation experiments**
- **Interactions can make cells different from each other: induction**



- In the process called induction
  - Signal molecules from embryonic cells cause transcriptional changes in nearby target cells

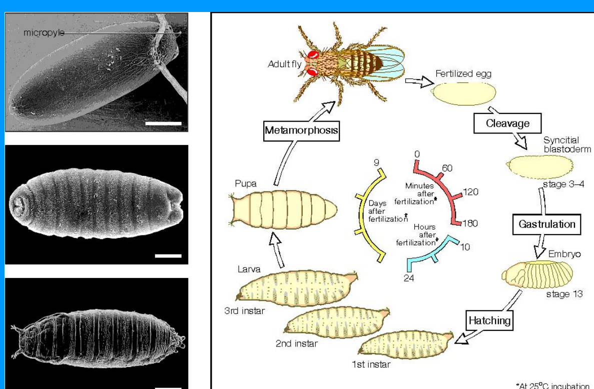


(b) Induction by nearby cells. The cells at the bottom of the early embryo depicted here are releasing chemicals that signal nearby cells to change their gene expression.

From single cell to organism – a life cycle

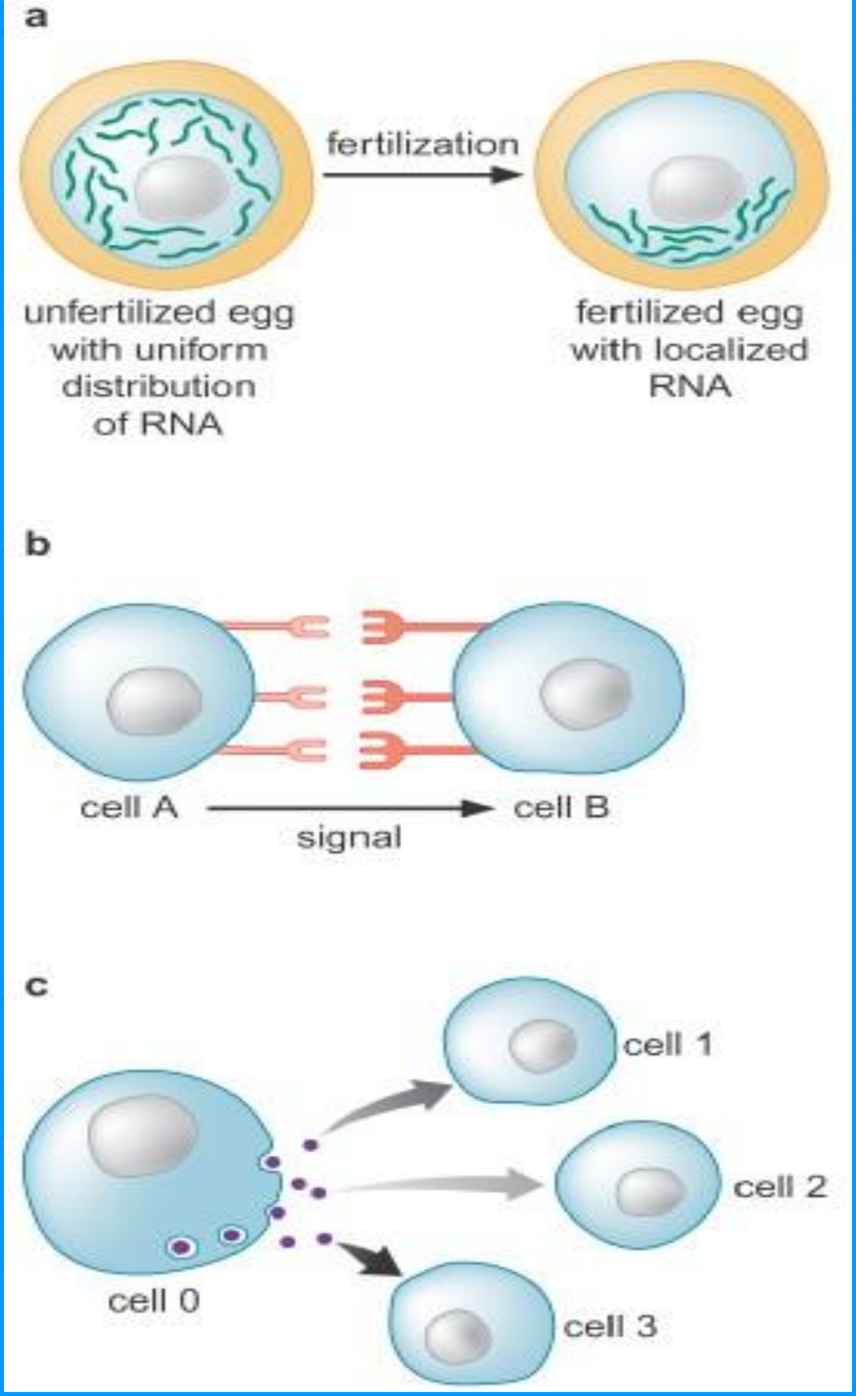
- Fertilisation followed by cell division
- Pattern formation – instructions for
  - Body plan (Axes: A-P, D-V)
  - Germ layers (ecto-, meso-, endoderm)
- Cell movement - form – gastrulation
- Cell differentiation
- Cell growth, cell death (apoptosis)

The use of a model organism



Three Strategies:

- **mRNA localization**
- **Cell-to-cell contact**
- **Signaling through the diffusion of secreted signaling molecules**




Место
встречи
изменить
нельзя

Морфогены
и рецепторы
морфогенов

- **Morphogen – substances that define different cell fates in a concentration-dependent manner**

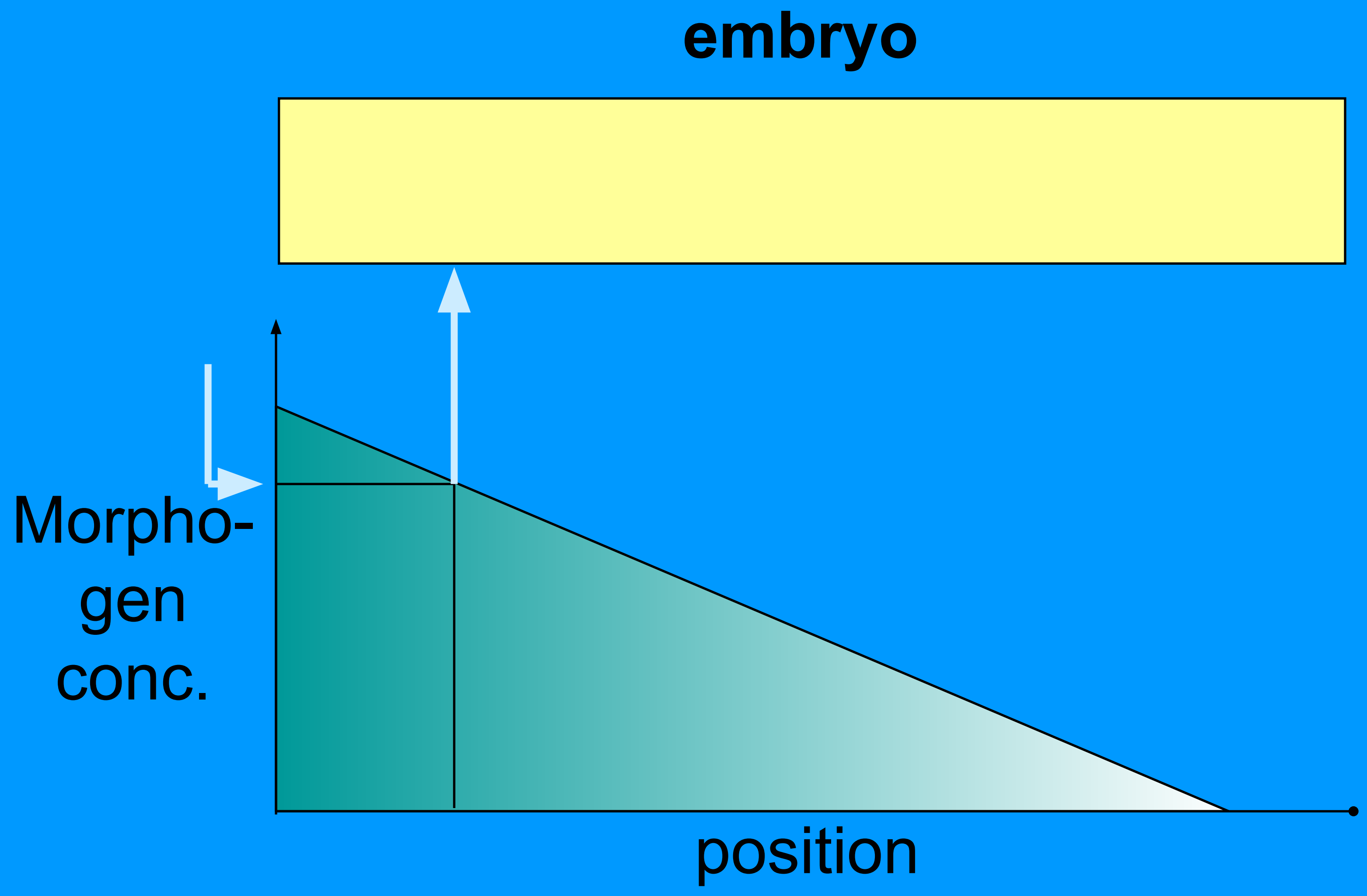
- **Interaction of two signaling centers located in the anterior and posterior poles of the egg pattern insect body axis**

- **Morphogen** = Soluble molecule that causes cellular commitment but is secreted some distance from the target cells.
- **Morphogen Gradient** = concentration

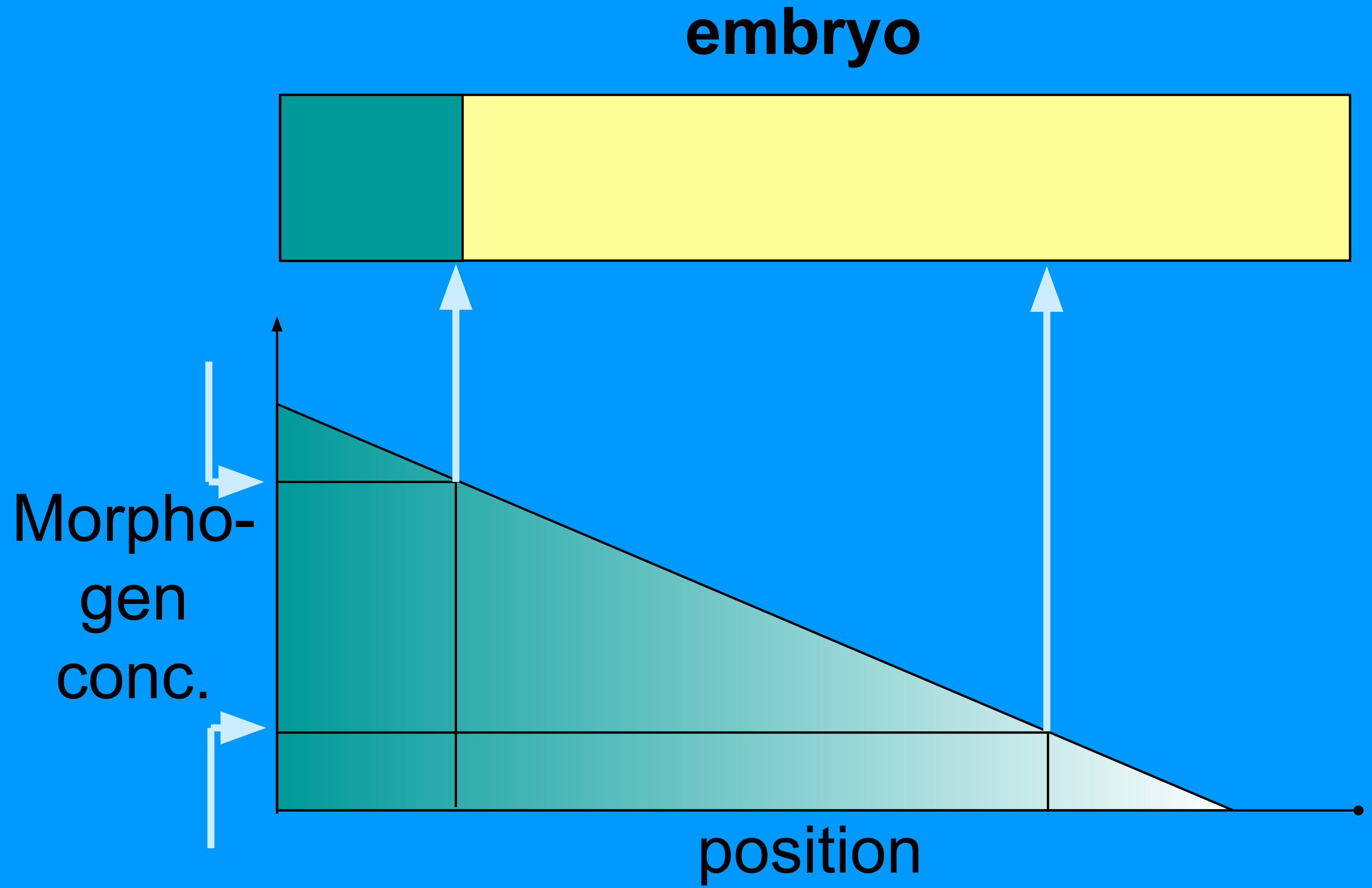
Градиенты морфогенов

- Белки, кодируемые генами, функционирующими в ходе созревания яйца, и транспортируемые туда из питающих клеток,
- распределяются по оси яйца,
- образуя градиенты, специфически характерные для продуктов каждого гена

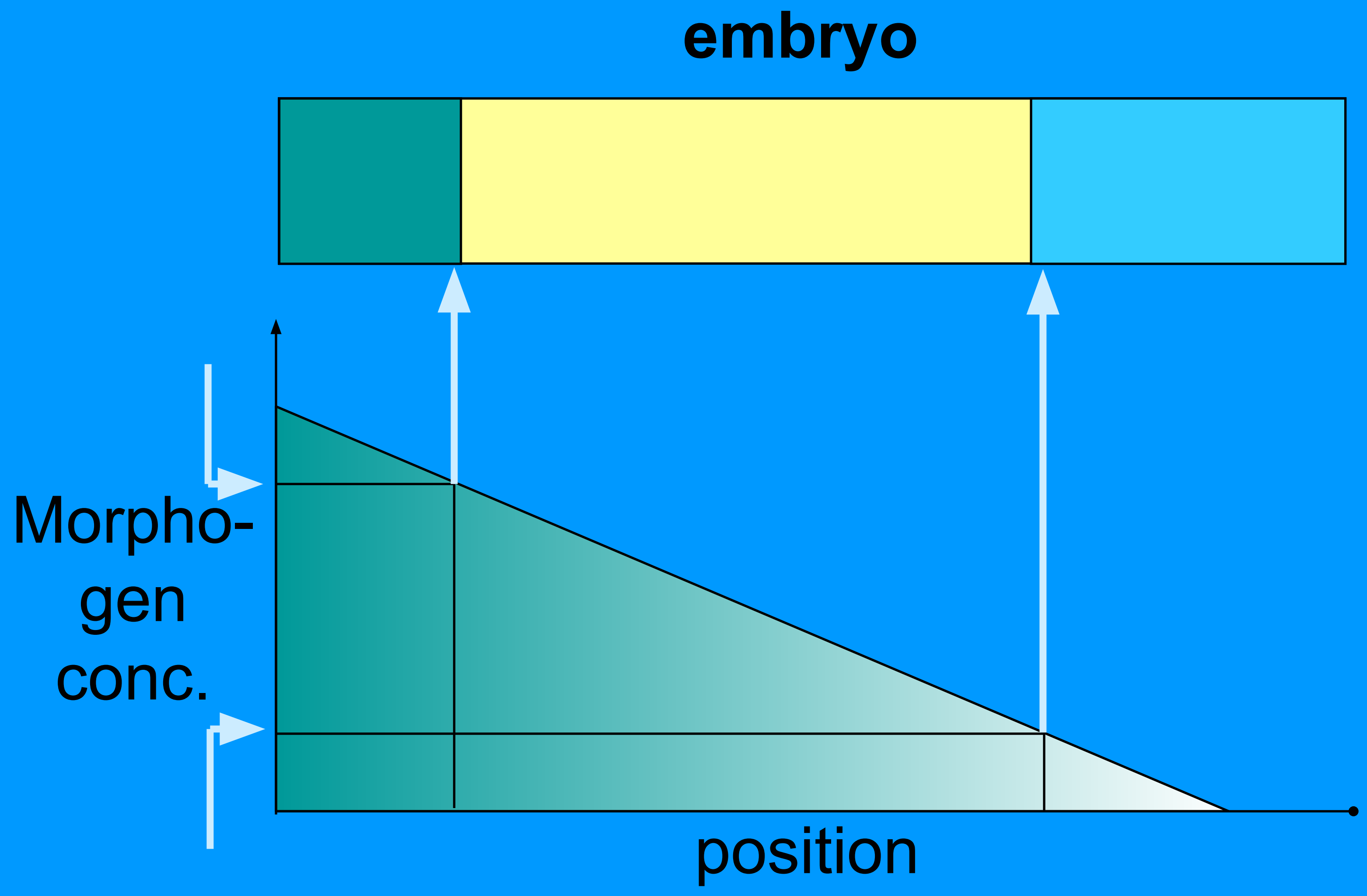
Morphogen Threshold Concentrations



Morphogen Threshold Concentrations



Morphogen Threshold Concentrations



Morphogen Receptor

Some mRNAs Become Localized within Eggs and Embryos due to an Intrinsic Polarity in the Cytoskeleton

- The asymmetrically distributed mRNA is transported along element of the cytoskeleton
- from – to the growing + end.

Adaptor protein

- The morphogene receptor binds to 3′untranslated trailer (3′UTR) region of morphogene mRNA,

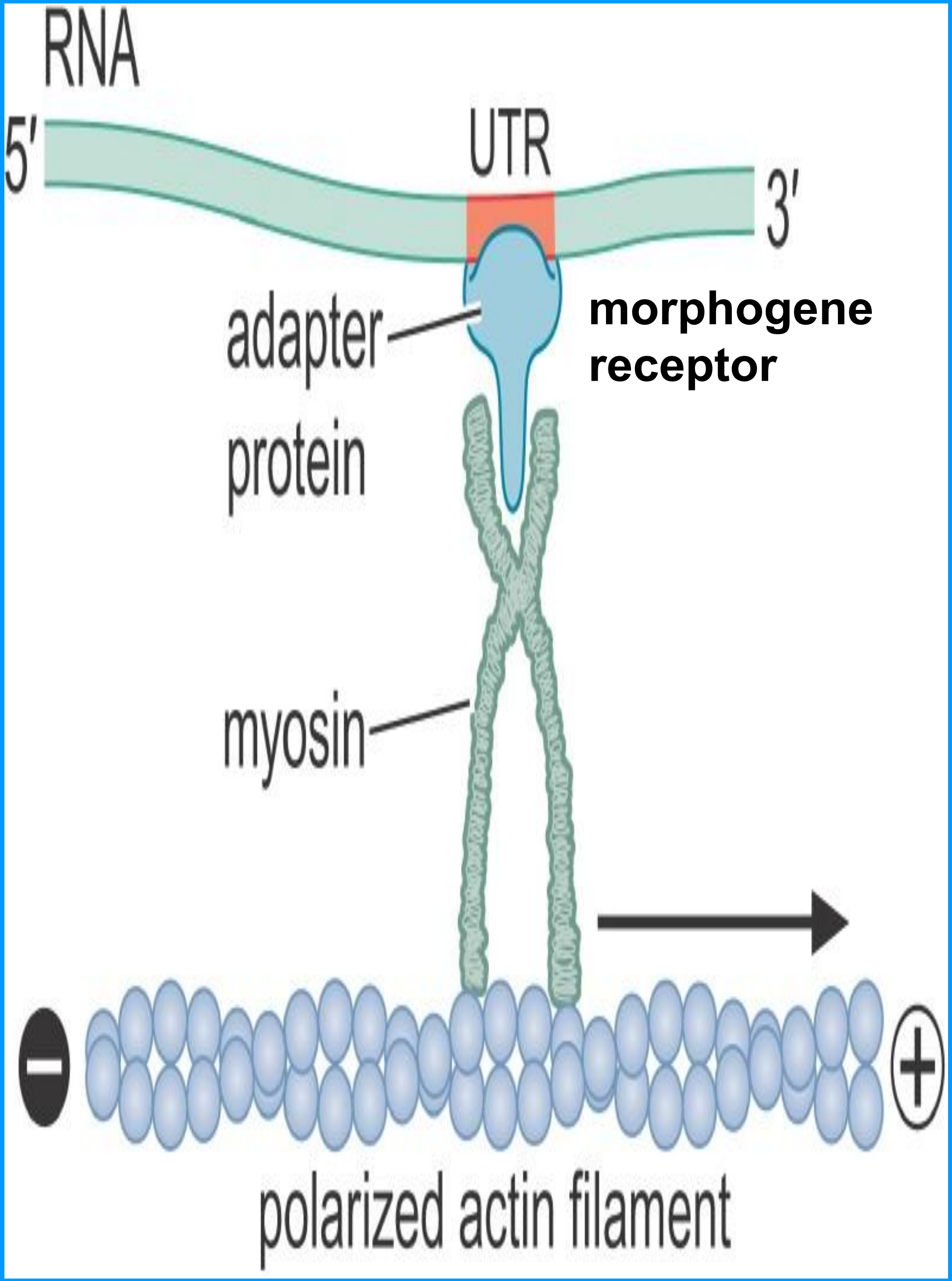
A morphogene receptor has two domains:

one recognizes the 3′ UTR,

the other

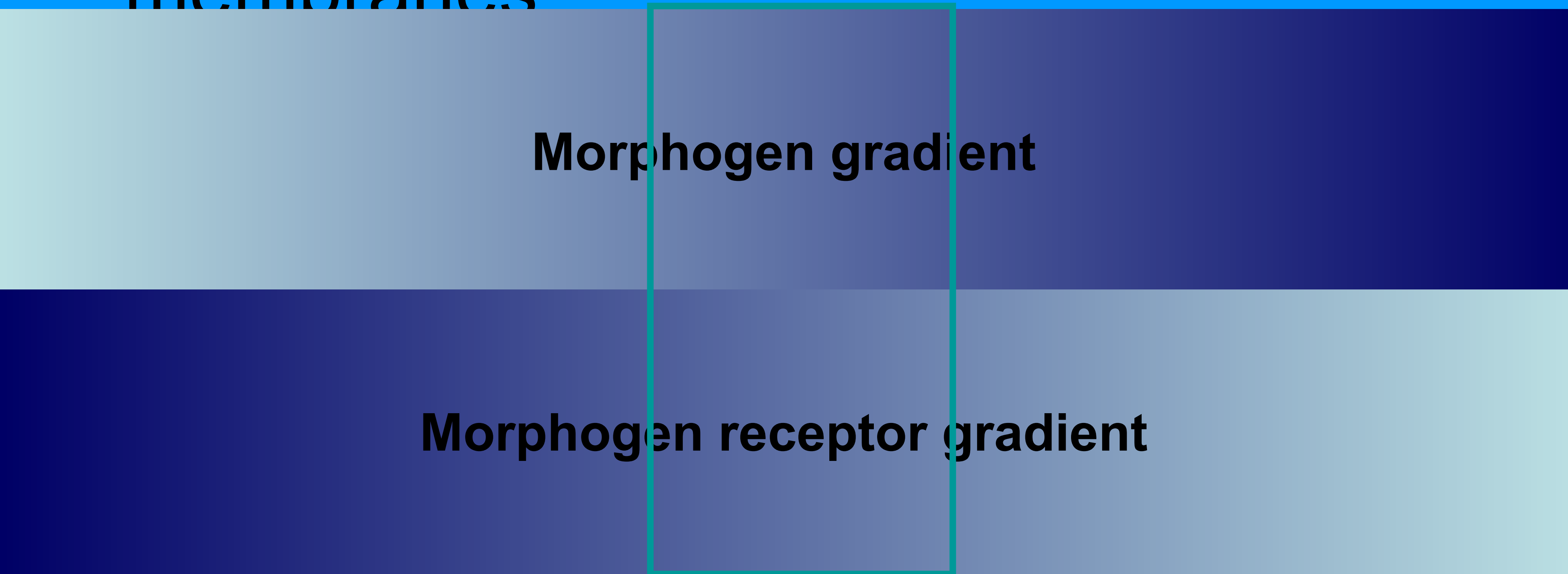
associates with myosin.

Cytoskeletal elements – microtubules are polarized within the oocyte and can be used to allow the localization of mRNA molecules to specific parts of the cell.



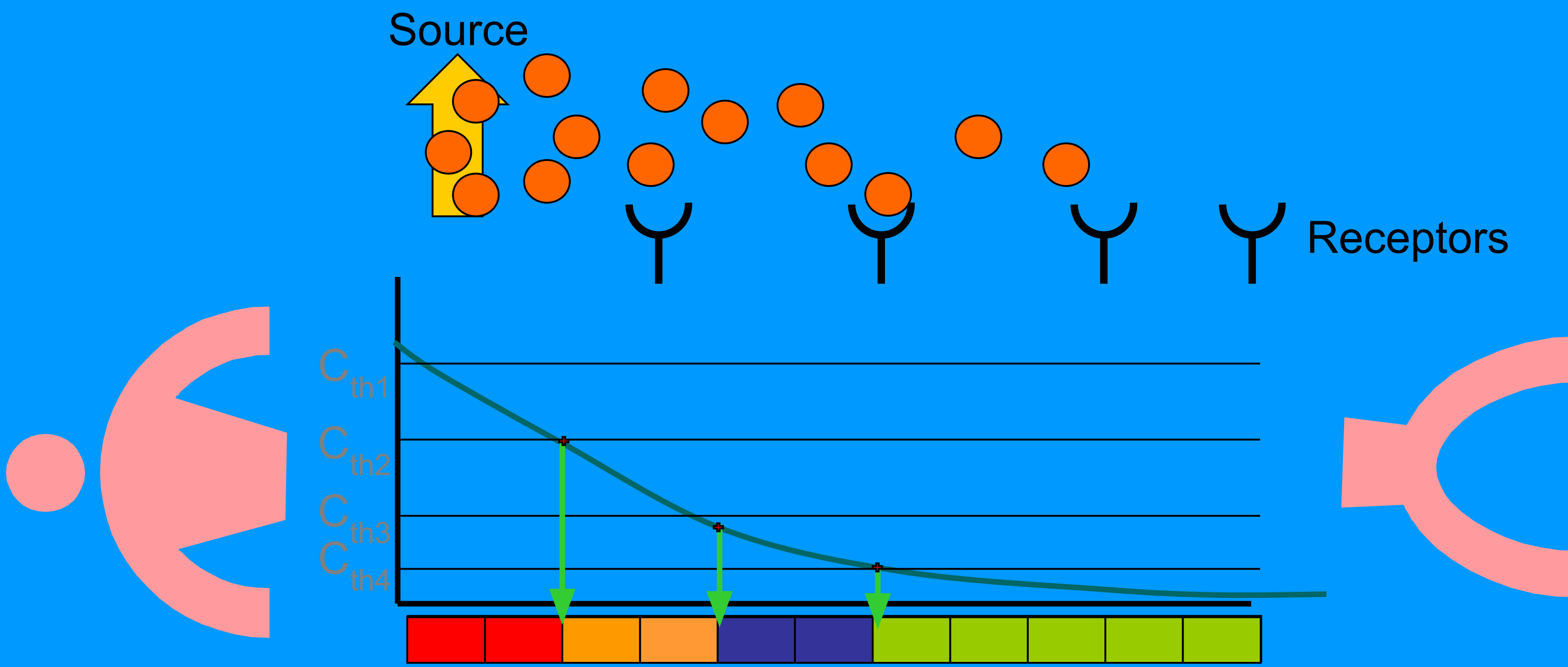
Influence of Other Cells

- **Morphogen Receptor Gradient** = frequency gradient of the receptors for a morphogen in target cell cell membranes

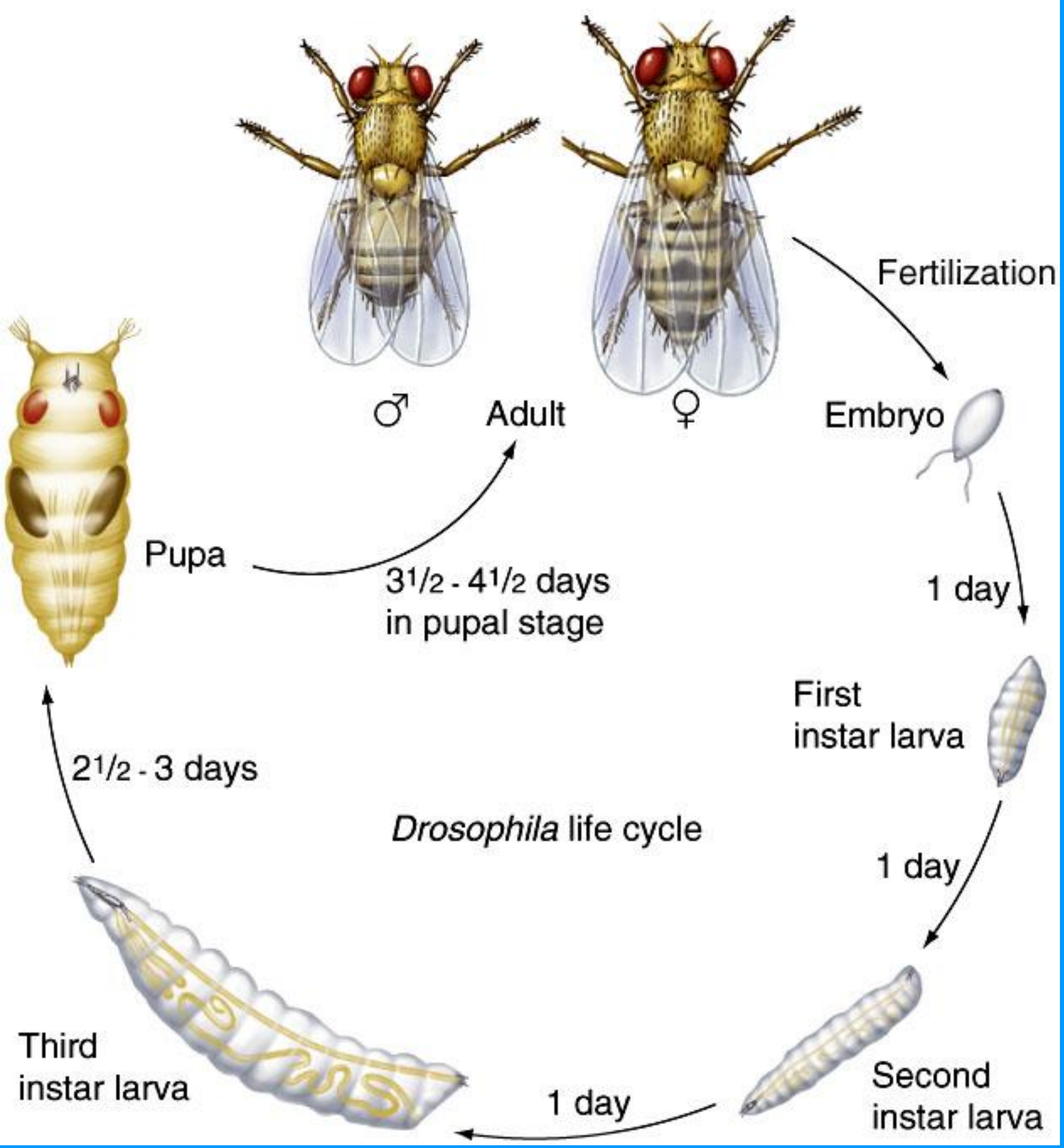


Morphogens

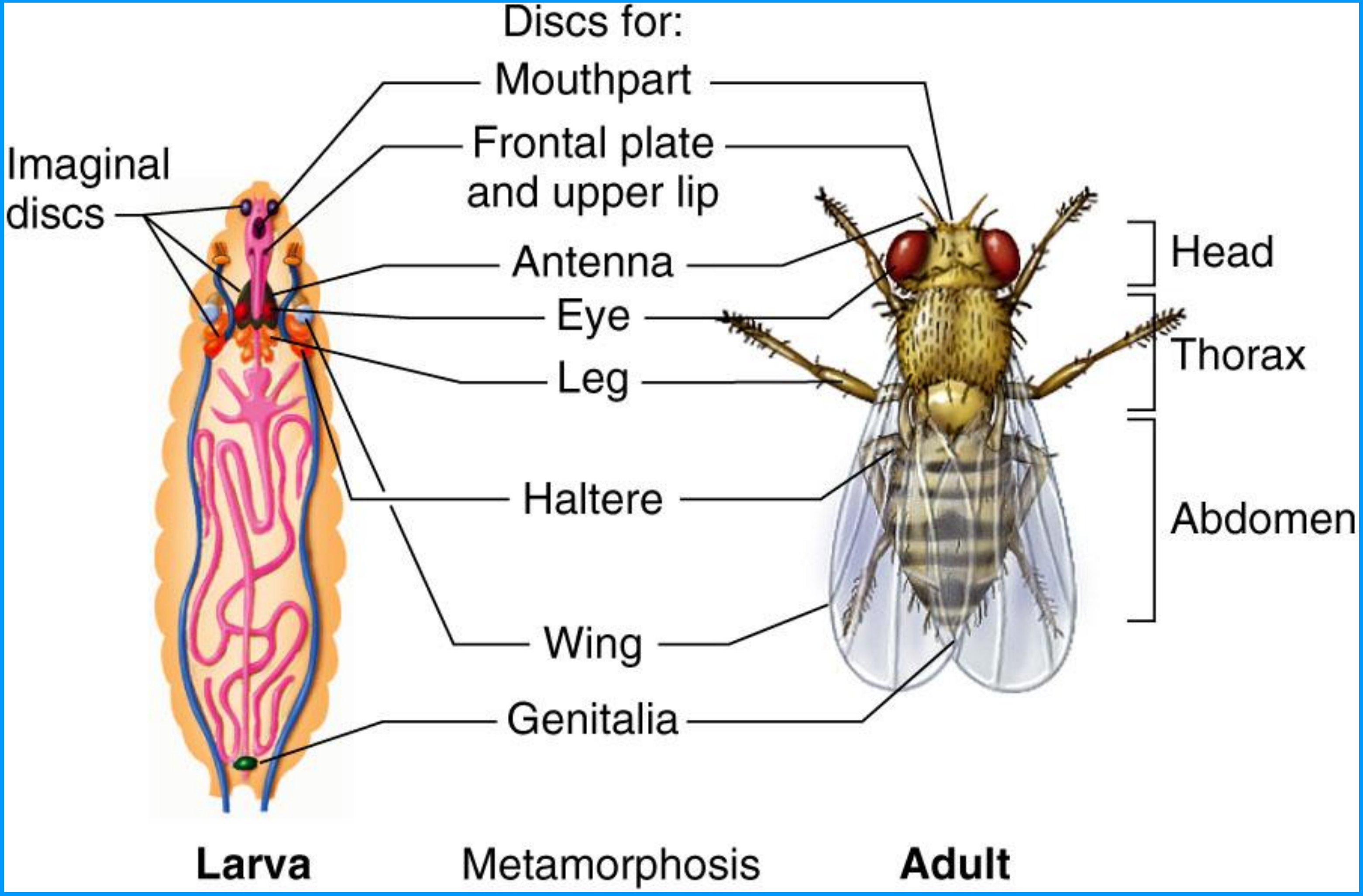
- Coordinate the cell growth and differentiation.
- Formation of a **long-range concentration profile**.
- Cellular response is concentration dependent.



Life cycle


Fertilization
♂ Adult ♀
Embryo
1 day
First instar larva
1 day
Second instar larva
1 day
Third instar larva
2 1/2 - 3 days
Pupa
3 1/2 - 4 1/2 days in pupal stage
Drosophila life cycle

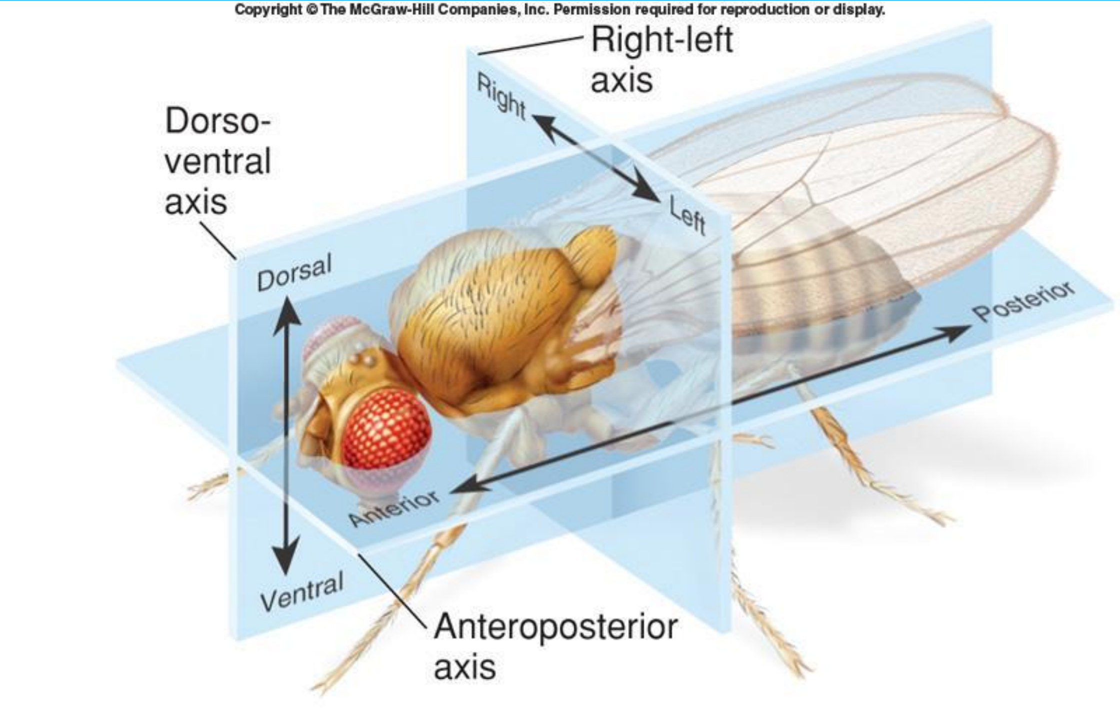
Many Adult Structures Develop from Imaginal Discs in Larvae and Pupae



Position or Spatial Organization is *Everything*

- **2 main mechanisms used to communicate positional information**

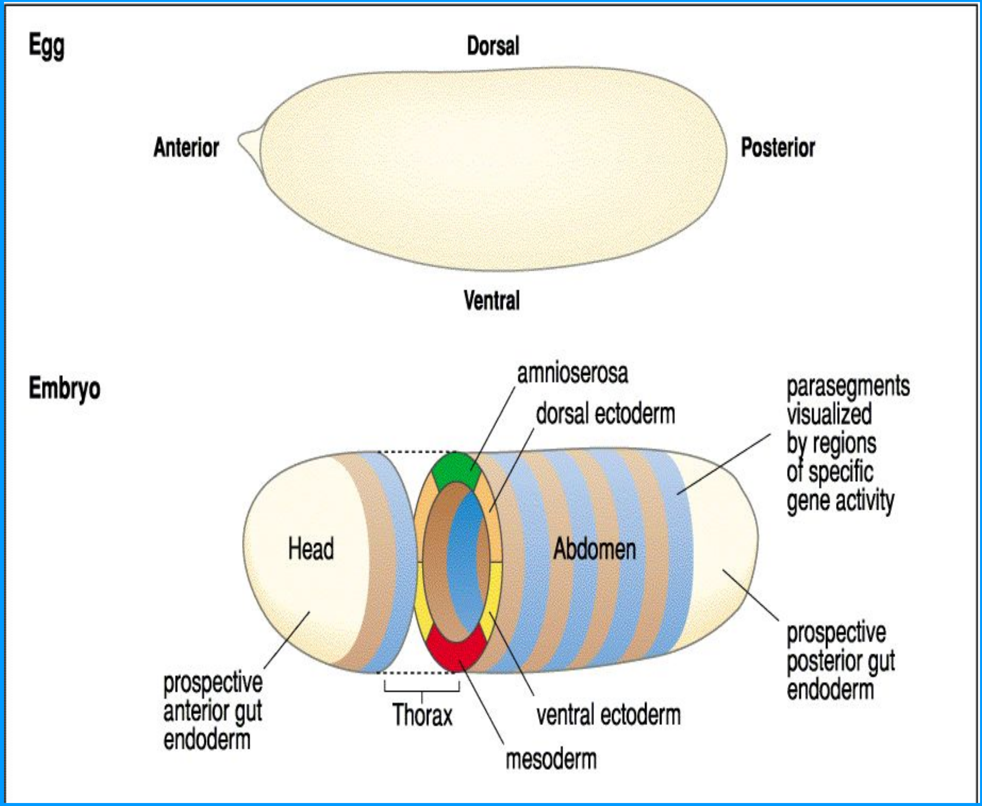
1. **Morphogens**
2. **Cell adhesion**

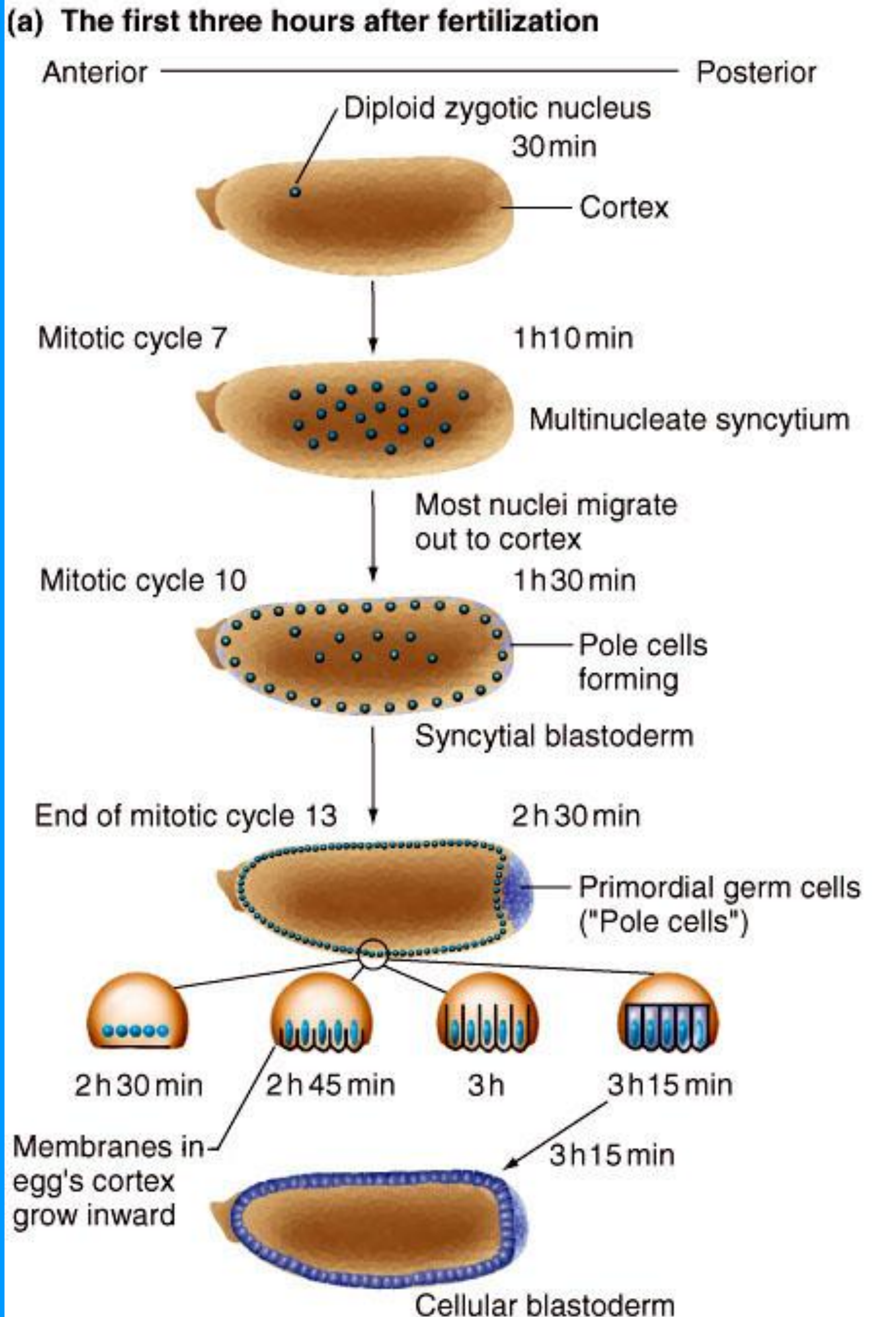


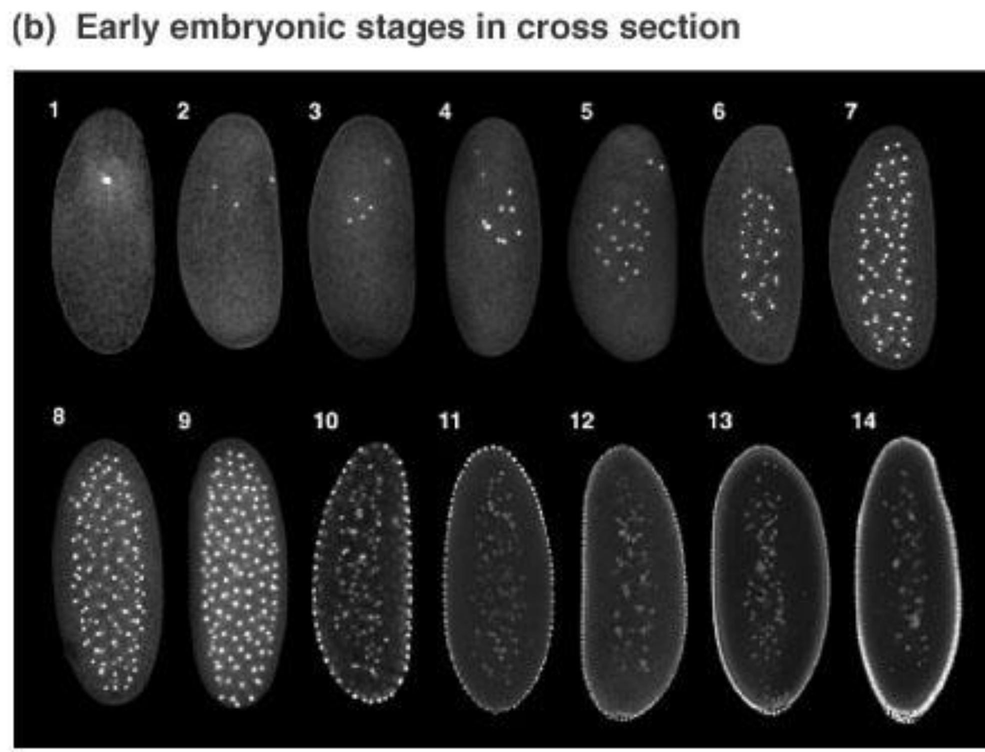
(a) Body plan found in many animals

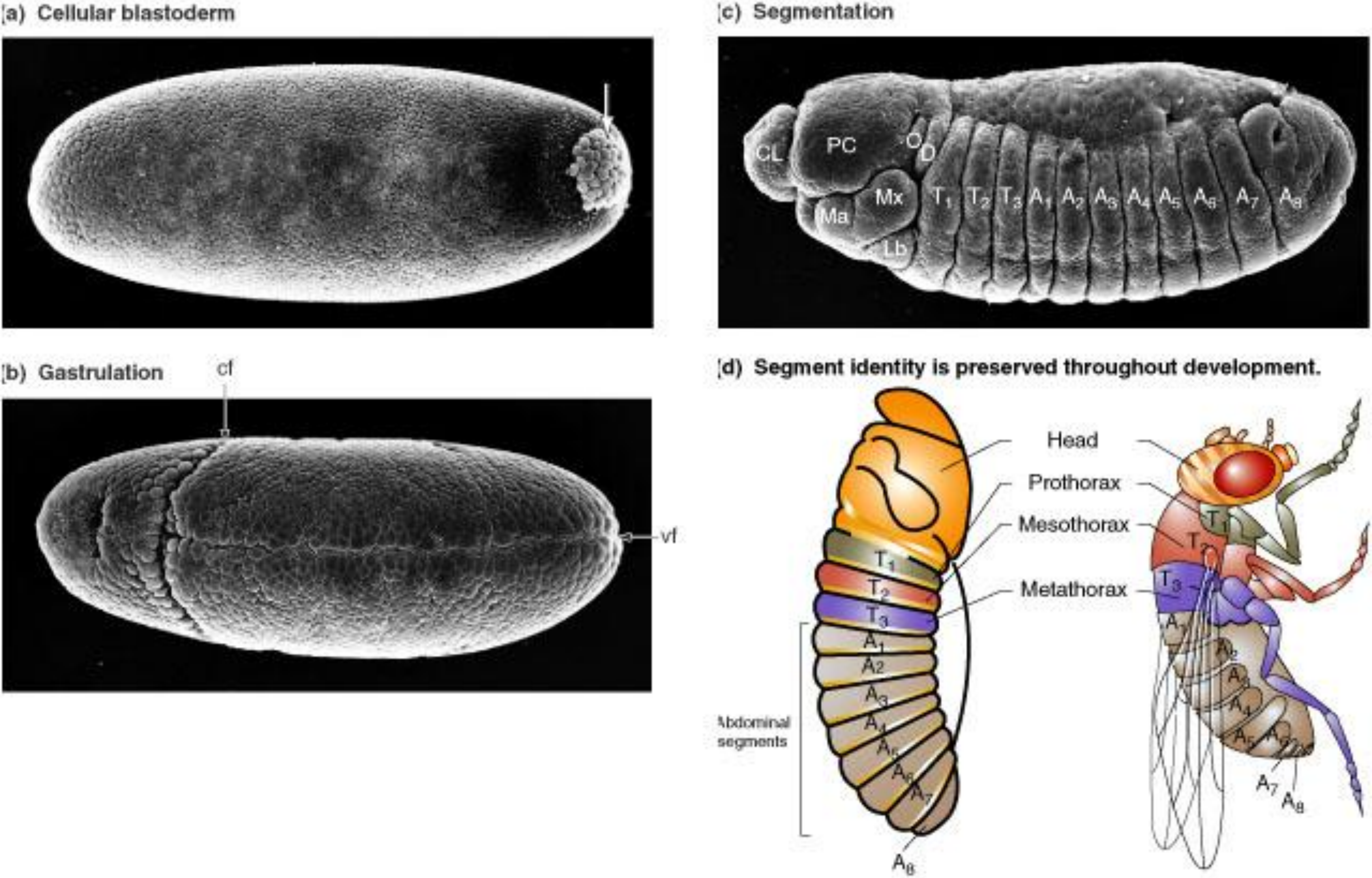
Fate Map: all different regions of an embryo develop is described

Developmental program — process for cell achieving its fate




(a) The first three hours after fertilization
Anterior
Posterior
Diploid zygotic nucleus
30 min
Cortex
Mitotic cycle 7
1 h 10 min
Multinucleate syncytium
Most nuclei migrate
out to cortex
Mitotic cycle 10
1 h 30 min
Pole cells
forming
Syncytial blastoderm
End of mitotic cycle 13
2 h 30 min
Primordial germ cells
("Pole cells")
2 h 30 min
2 h 45 min
3 h
3 h 15 min
Membranes in
egg's cortex
grow inward
3 h 15 min
Cellular blastoderm


(b) Early embryonic stages in cross section
1
2
3
4
5
6
7
8
9
10
11
12
13
14


(a) Cellular blastoderm
(b) Gastrulation
cf
vf
(c) Segmentation
CL
PC
C
D
Mx
Ma
Lb
T1 T2 T3 A1 A2 A3 A4 A5 A6 A7 A8
(d) Segment identity is preserved throughout development.
Head
Prothorax
Mesothorax
Metathorax
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
Abdominal segments

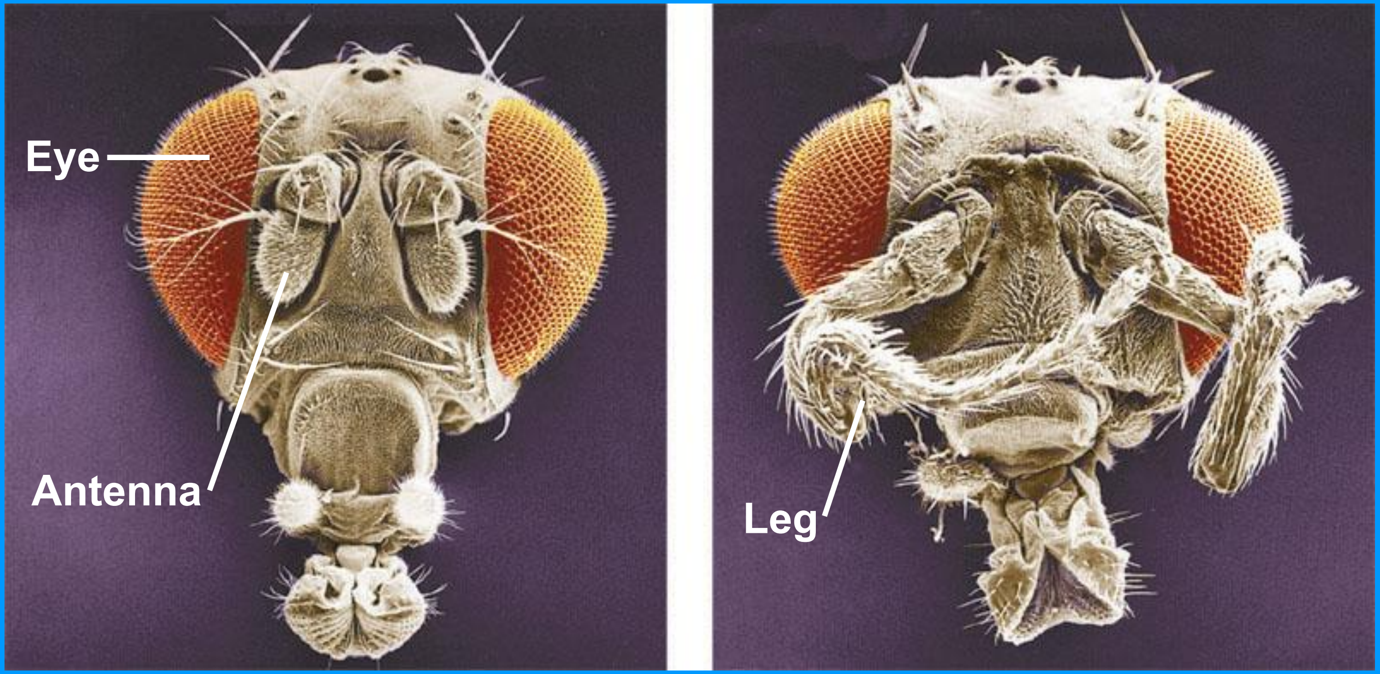
- **Морфогены активируют**

- **Гены сегментации активируют**

- **Гомеозисные гены активируют…**

Genetic Analysis of Early Development: Scientific Inquiry

- The study of developmental mutants
  - Laid the groundwork for understanding the mechanisms of development



Wild type Mutant

Drosophila Embryogenesis

First phase is establishment of body axes

Before fertilization, specialized nurse cells

move maternal mRNAs (morphogenes) into maturing oocyte

Then morphogens are distributed in the oocyte prior to fertilization

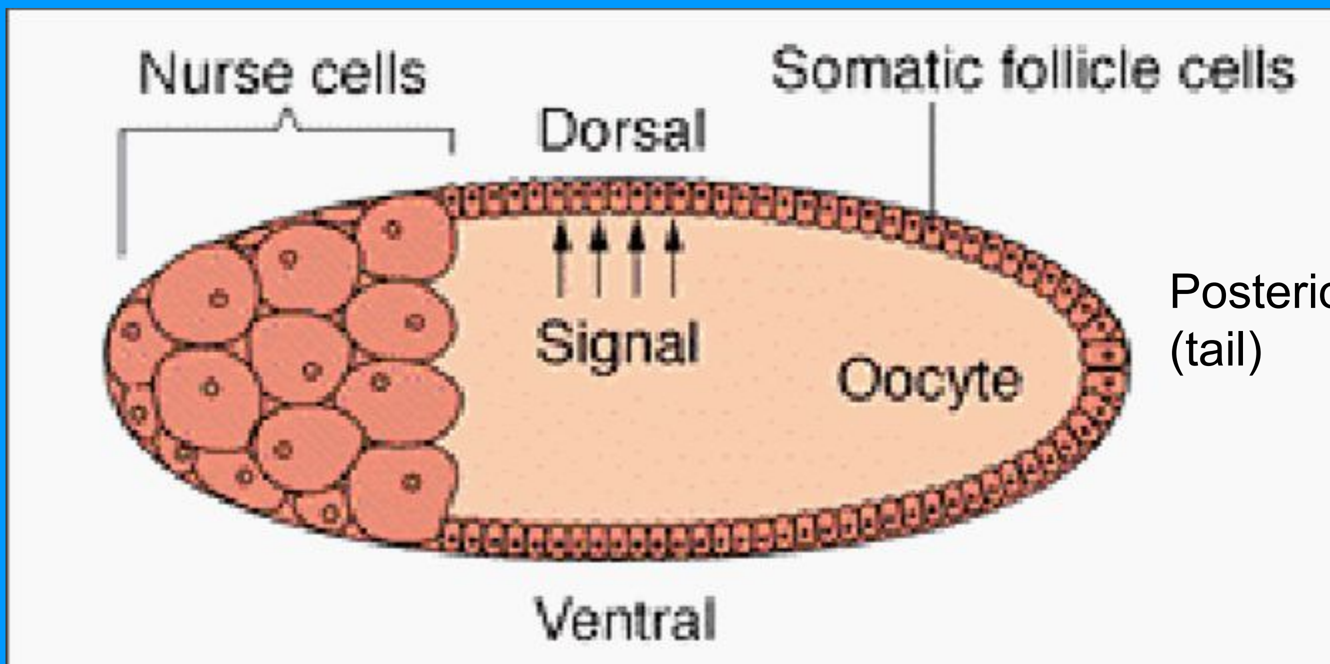
- **The Morphogens organize the structure of the egg**

These positioned mRNA will initiate a cascade
of gene activations following fertilization

- **Яйцо созревает в особой камере — фолликуле.**
- **В фолликулах - гены с материнским эффектом, которые функционируют еще до оплодотворения яйца сперматозоидом**
- **В фолликуле ооцит и 15 огромных питающих клеток,**
- **которые синтезируют «РНК морфогены» и перекачивают их в ооцит.**

(top)

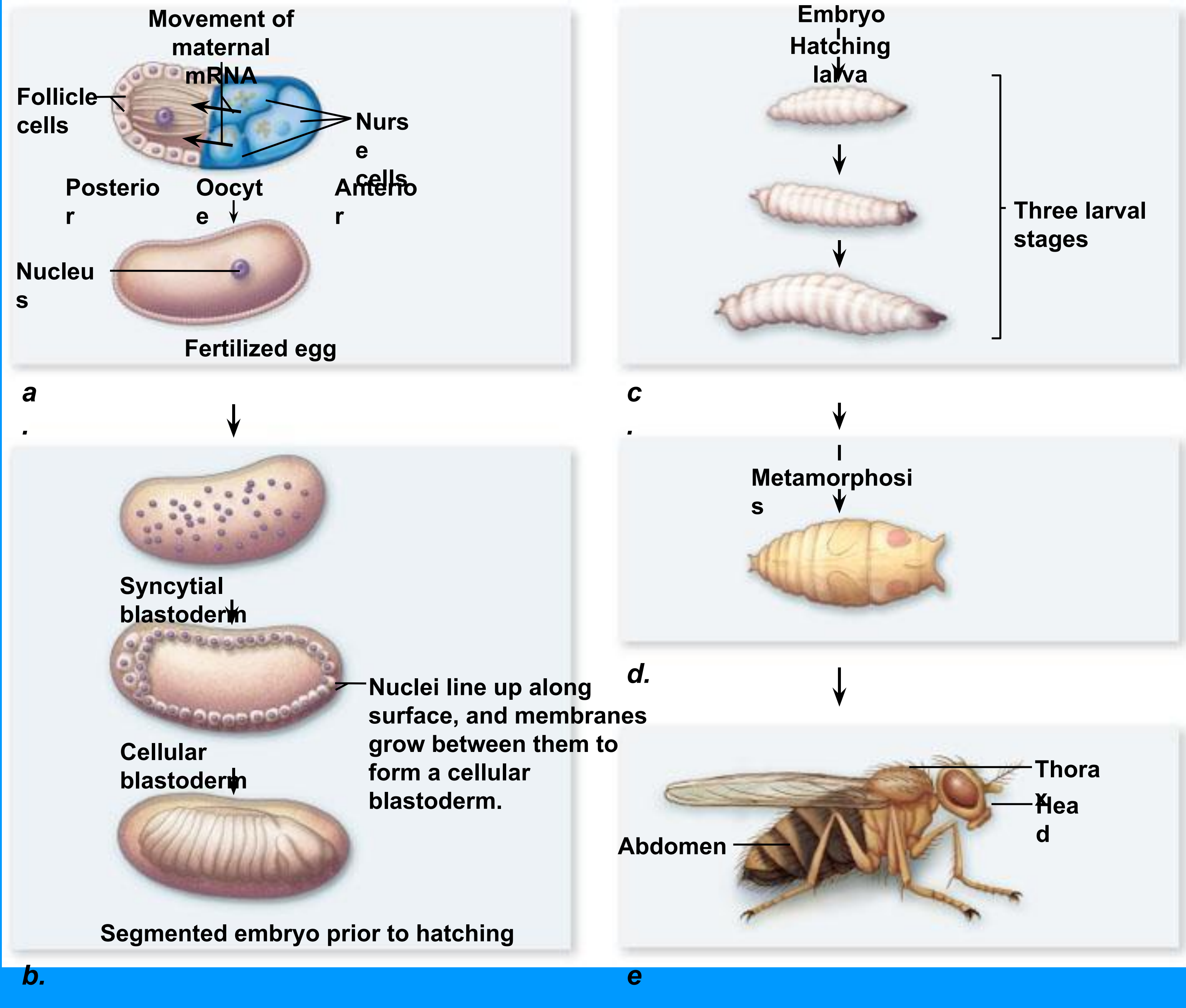
Anterior
(head)



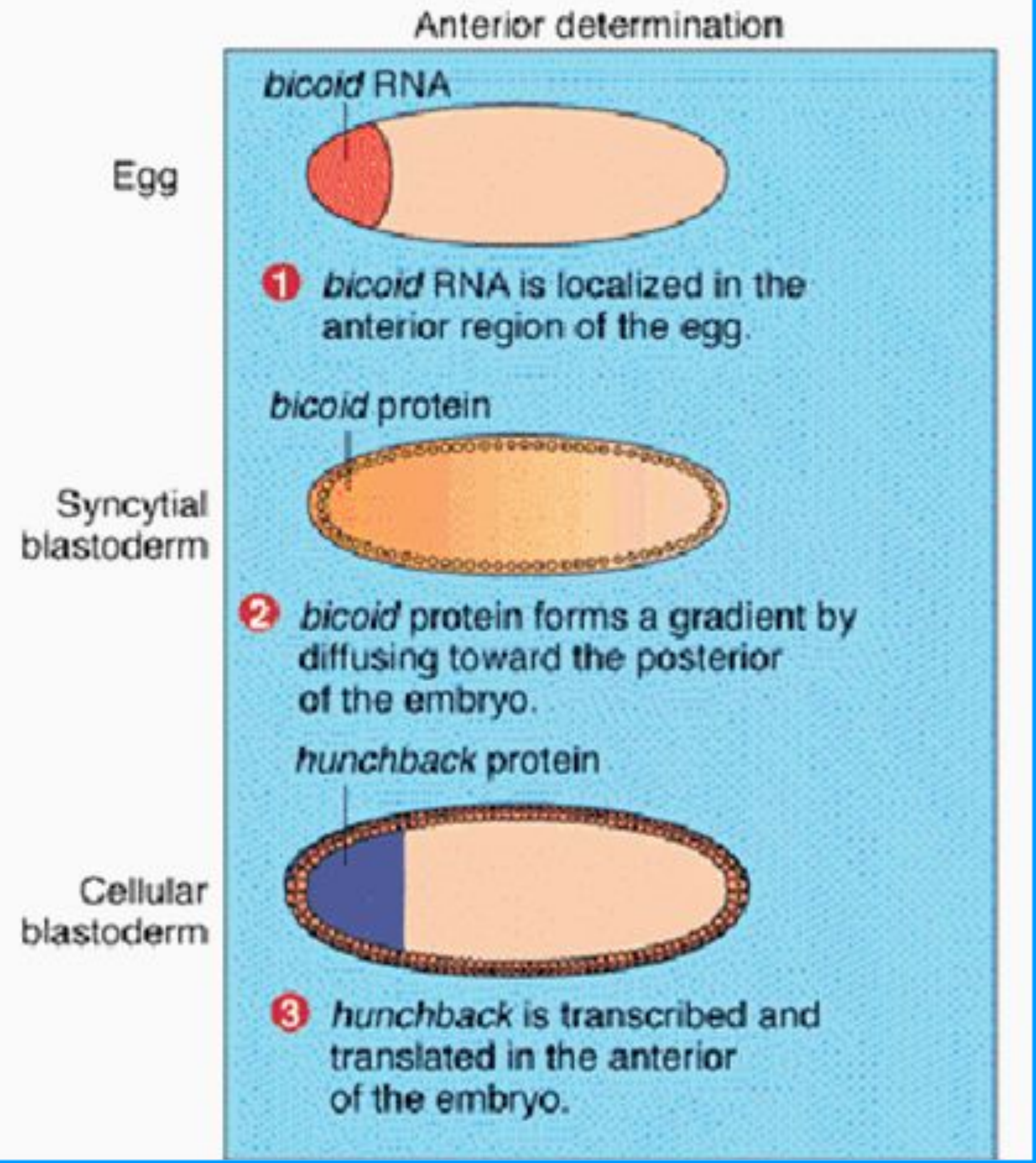
Posterior
(tail)

(underneath)

At the start of development, ***gradients*** are established in the egg along two axes, ***anterior-posterior*** and ***dorsal-ventral***.



Movement of maternal mRNA
Follicle cells
Nurs
e
cells
Posterio
r
Oocyt
e
Anterio
r
Nucleu
s
Fertilized egg
a
.
Syncytial blastoderm
Nuclei line up along surface, and membranes grow between them to form a cellular blastoderm.
Cellular blastoderm
Segmented embryo prior to hatching
b.
Embryo
Hatching larva
Three larval stages
c
.
Metamorphosi
s
d.
Thora
x
Hea
d
Abdomen
e



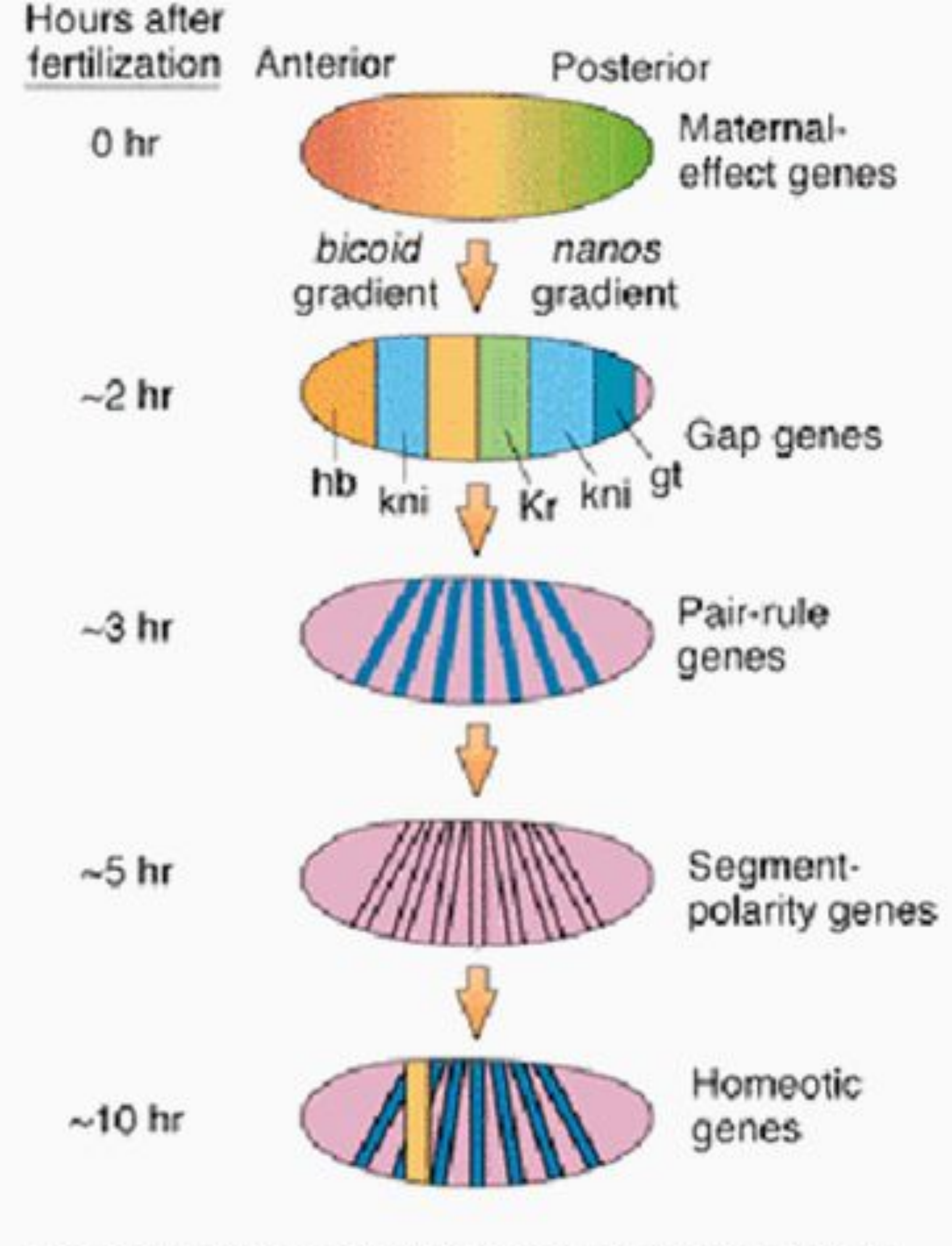
1. Maternal gene products, called ***morphogens***, establish gradients in early embryogenesis.

2. Anterior-posterior development uses localized gene regulators.

3. Dorsal-ventral development uses localized receptor-ligand interactions.

Градиент морфогенов активирует зиготные гены

- <u>После того</u> как градиенты в яйце созданы, происходит оплодотворение и начинается дробление зародыша
- образуется однослойная бластодерма. Каждая клетка в ней занимает определенное положение по отношению к сформировавшимся градиентам,
- - обладает определенной позиционной информацией.
- Морфогены взаимодействуют с регуляторными участками генов, активирующихся у зигот (зиготических генов).



***Expressed during oogenesis by the mother*. Act upon or within the maturing oocyte.**

***Expressed after fertilization*. Mutations in these genes alter the number or polarity of segments. Three groups of segmentation genes act sequentially to define increasingly smaller regions of the embryo.**

***Control the identity of a segment*, but do not affect the number, polarity or size of segments. Mutations in these genes cause one body part to develop the phenotype of another part.**

Establishment of the A/P axis

Nurse cells secrete maternally produced *bicoid* and *nanos* mRNAs into the oocyte

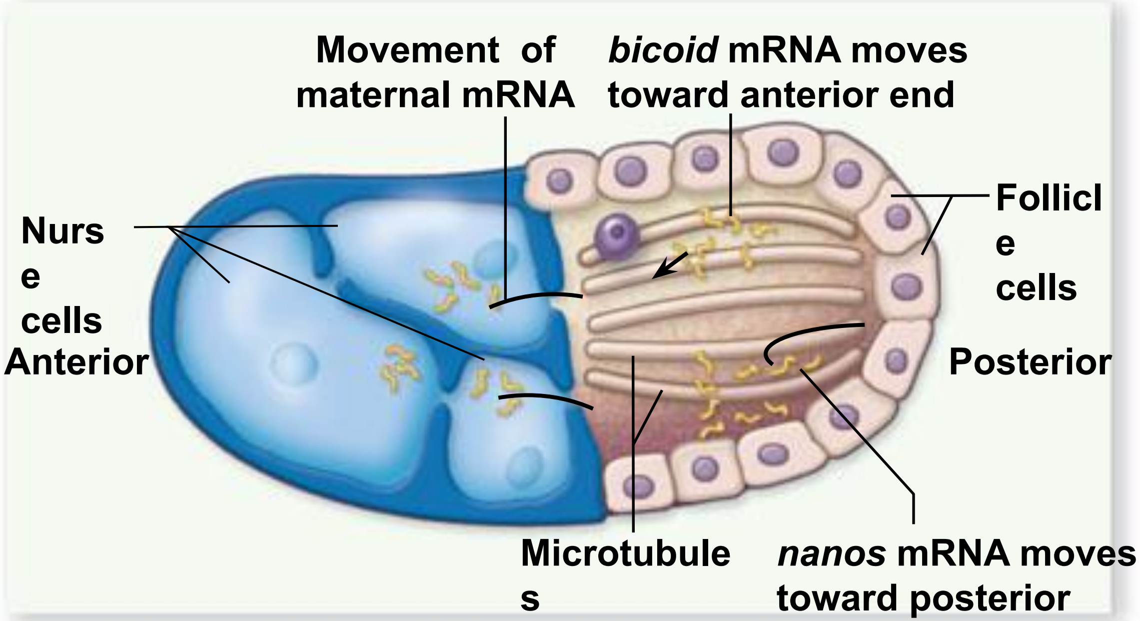
-Differentially transported by microtubules to opposite poles of the oocyte

-*bicoid* mRNA to the future anterior pole

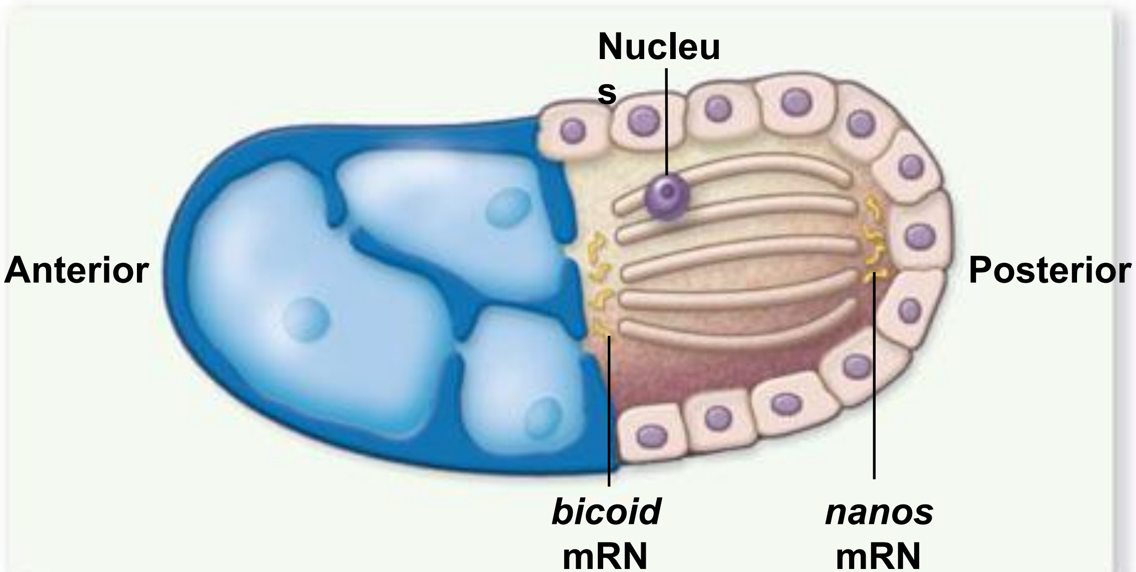
-*nanos* mRNA to the future posterior pole

-After fertilization, translation will create opposing gradients

of **Bicoid** and **Nanos** proteins


Movement of maternal mRNA
bicoid mRNA moves toward anterior end
Nurs e cells
Anterior
Follicl e cells
Posterior
Microtubule s
nanos mRNA moves toward posterior end

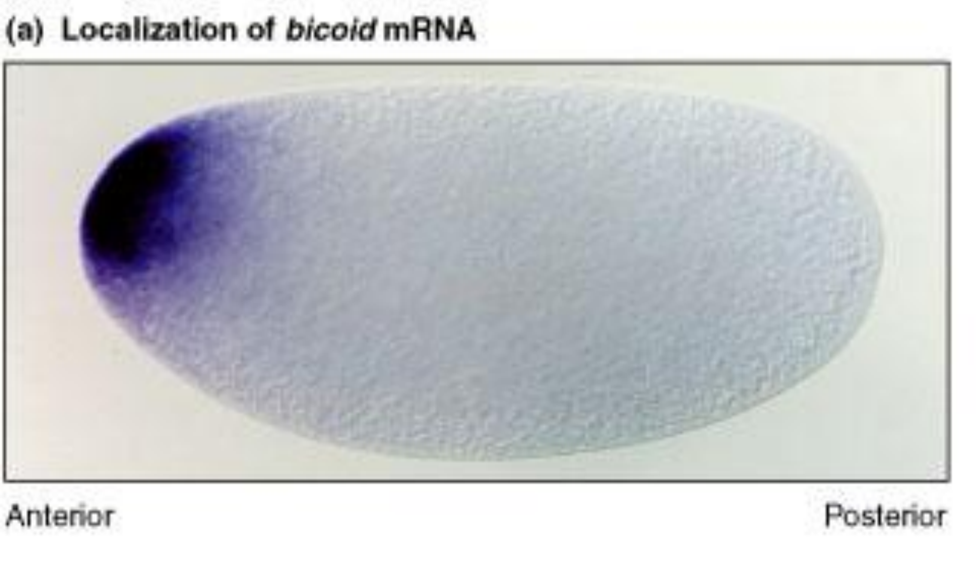
a.

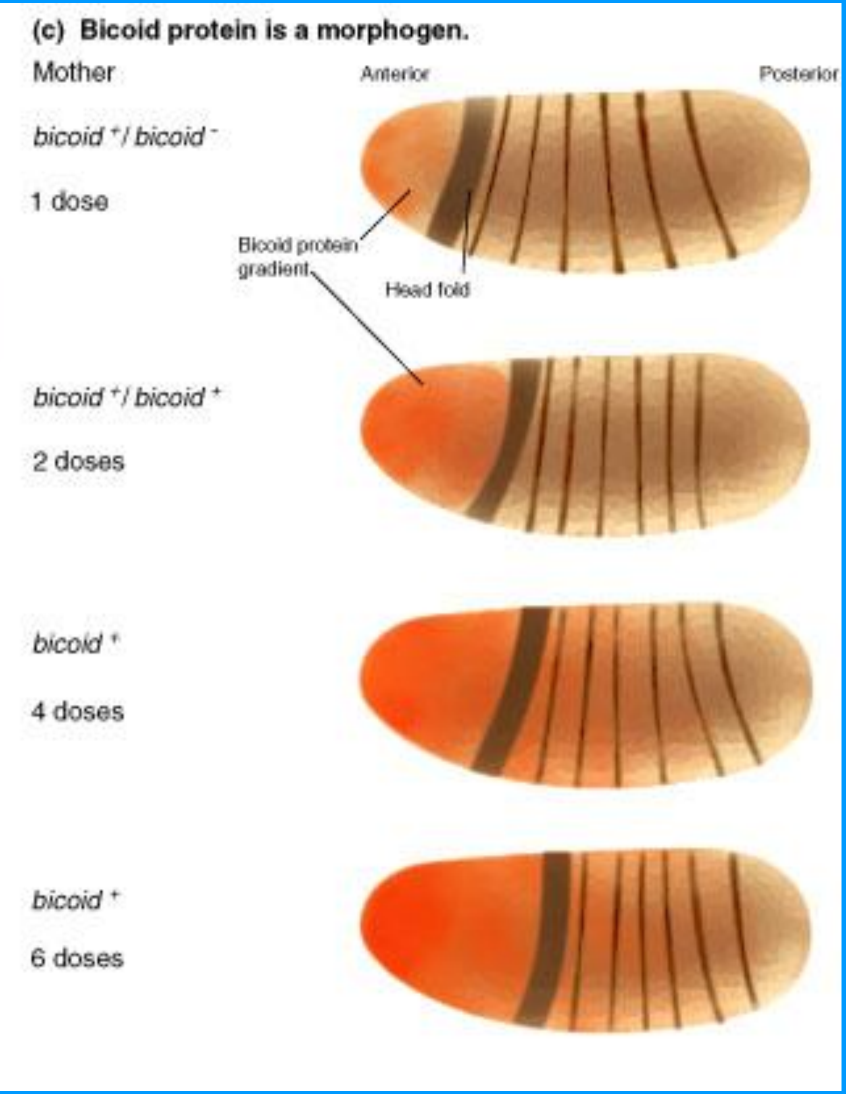

Nucleu s
Anterior
Posterior
bicoid mRN A
nanos mRN A

b.

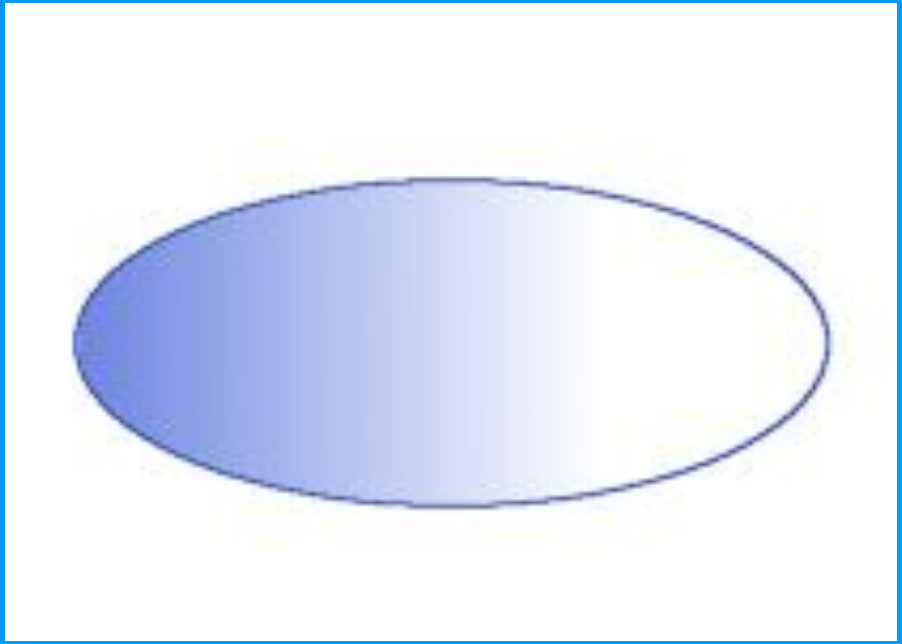
bicoid (*bcd*) Encodes the Anterior Morphogen

•



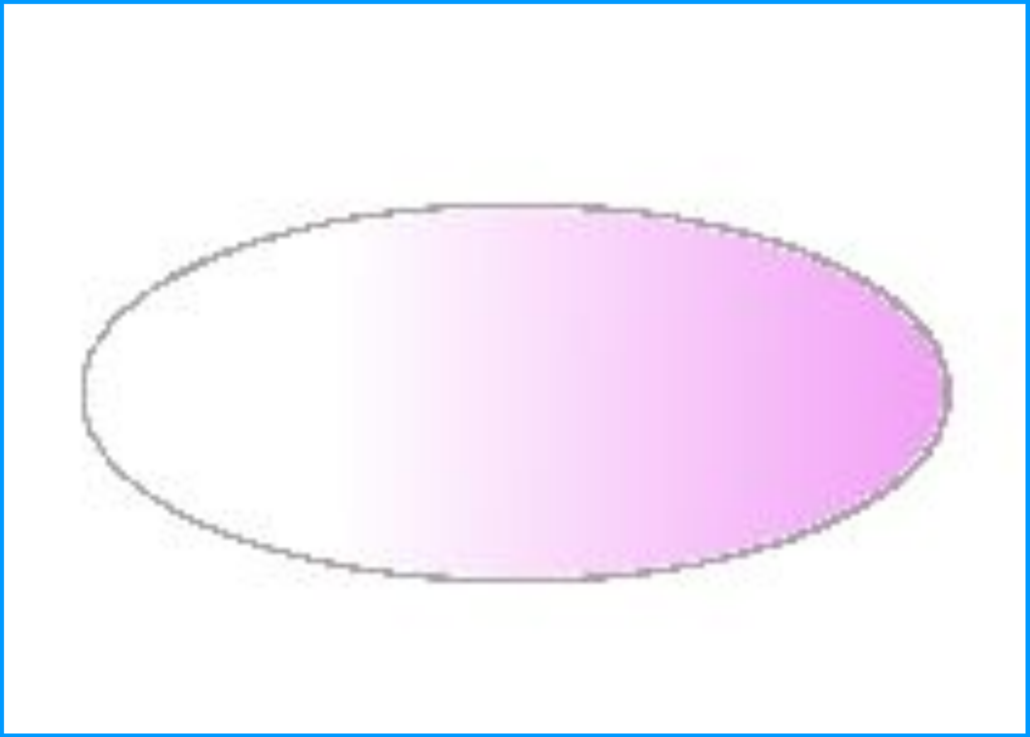


Bicoid gradient



- **Bicoid acts as a transcriptional activator of *hunchback* gene transcription**

Nanos gradient



Establishment of the A/P axis

Bicoid and Nanos control translation of two other maternal mRNAs, *hunchback* and *caudal*, that encode transcription factors

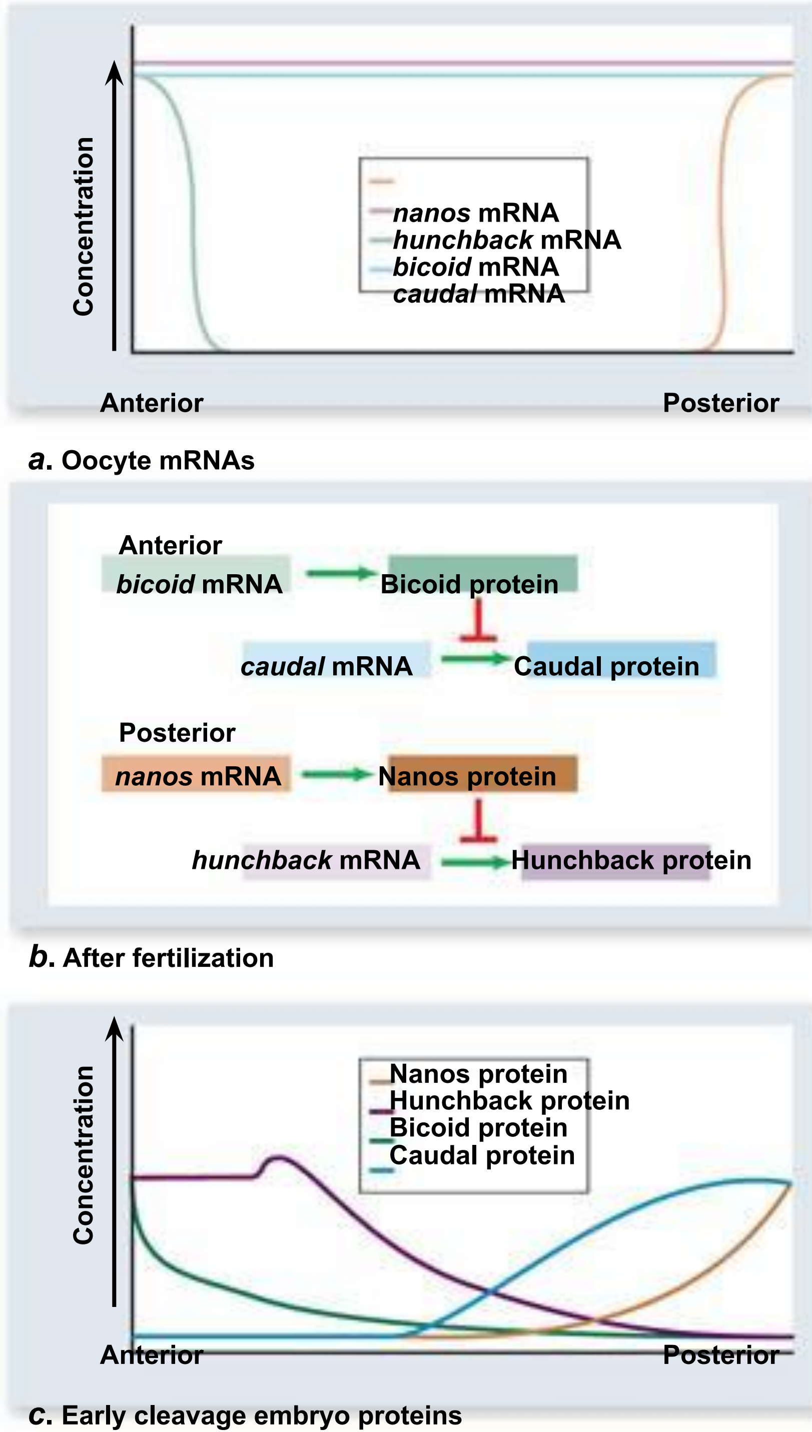
-**Hunchback** activates anterior structures

-**Caudal** activates posterior structures

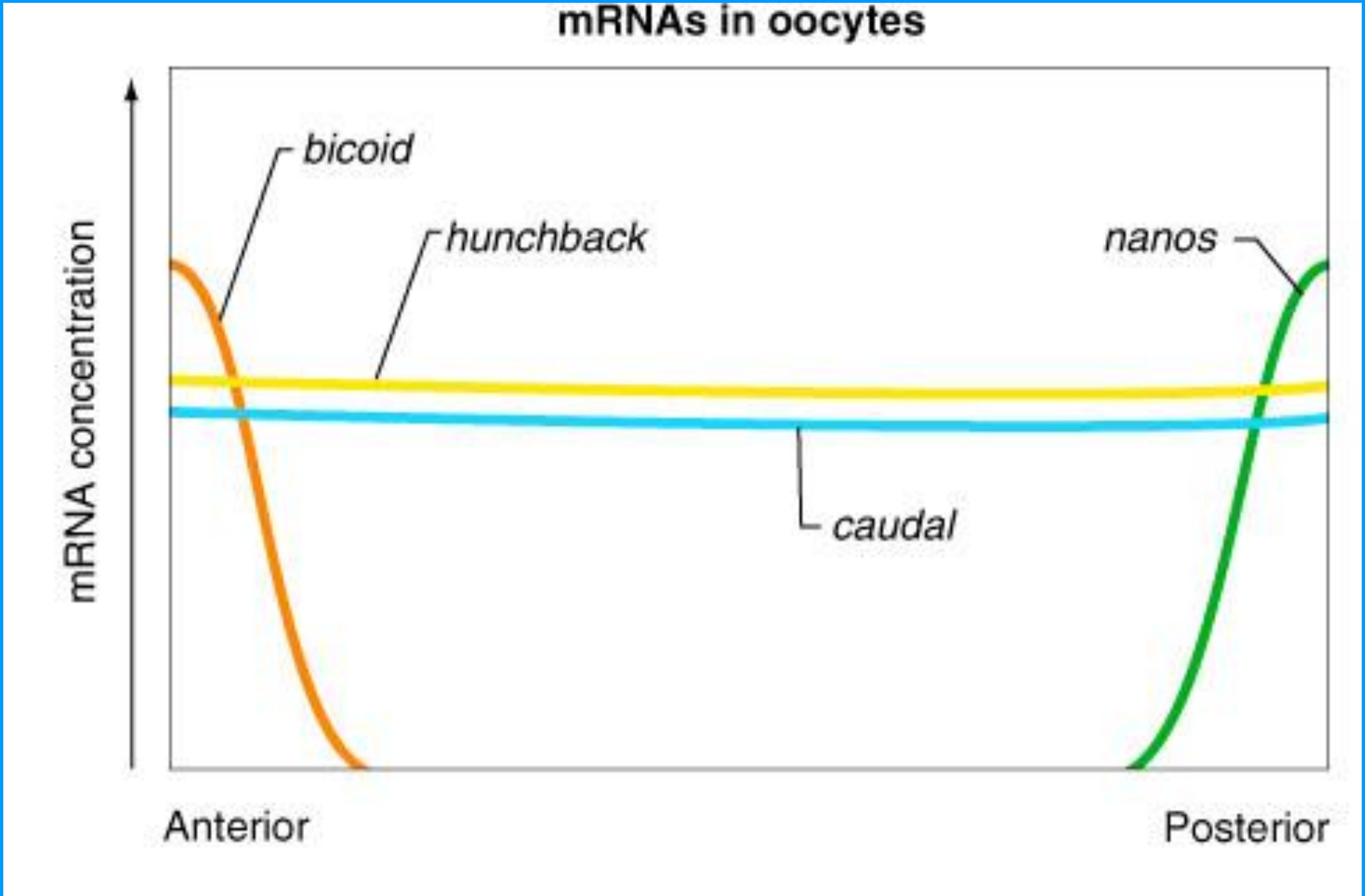
The two mRNAs are not evenly distributed

-Bicoid protein inhibits *caudal* mRNA translation

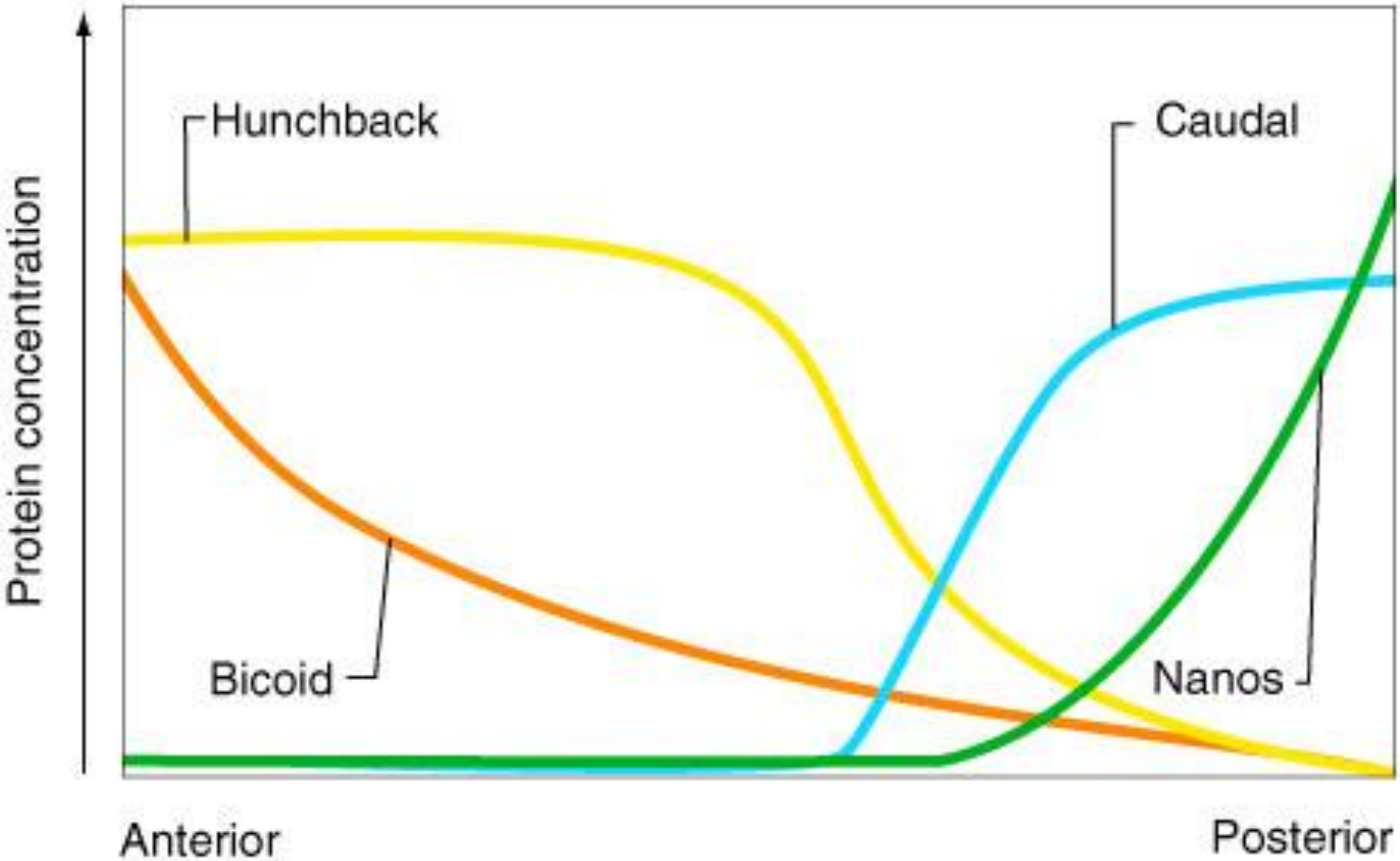
-Nanos protein inhibits *hunchback* mRNA translation


Concentration
nanos mRNA
hunchback mRNA
bicoid mRNA
caudal mRNA
Anterior
Posterior
a. Oocyte mRNAs
Anterior
bicoid mRNA
Bicoid protein
caudal mRNA
Caudal protein
Posterior
nanos mRNA
Nanos protein
hunchback mRNA
Hunchback protein
b. After fertilization
Concentration
Nanos protein
Hunchback protein
Bicoid protein
Caudal protein
Anterior
Posterior
c. Early cleavage embryo proteins

How Bcd Protein Works



Proteins in early cleavage embryos



Establishment of the D/V axis

Maternally produced ***dorsal* mRNA**

is placed into the oocyte

- -Not asymmetrically localized

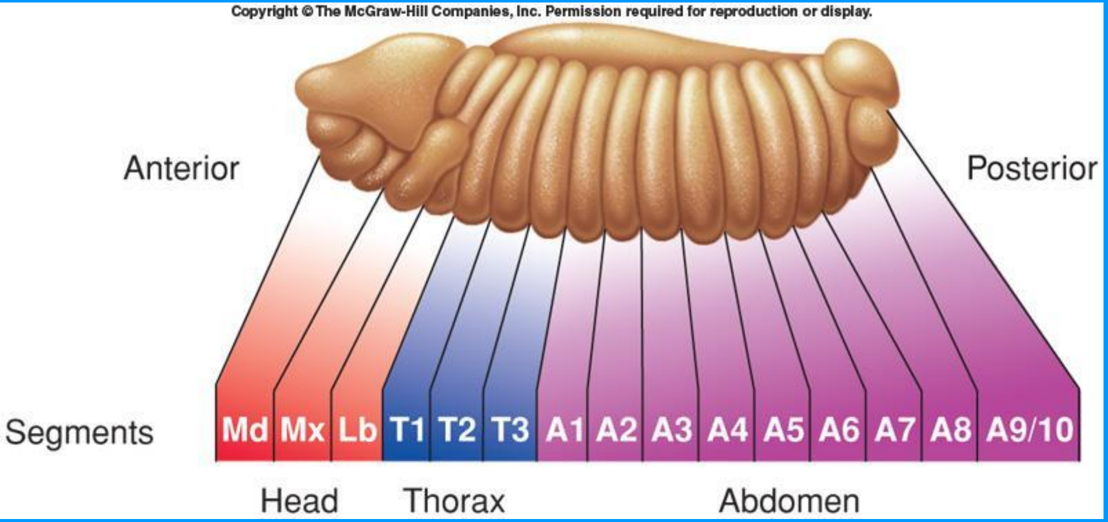
Oocyte nucleus synthesizes *gurken* mRNA

- -Accumulates in a crescent on the future dorsal side of embryo

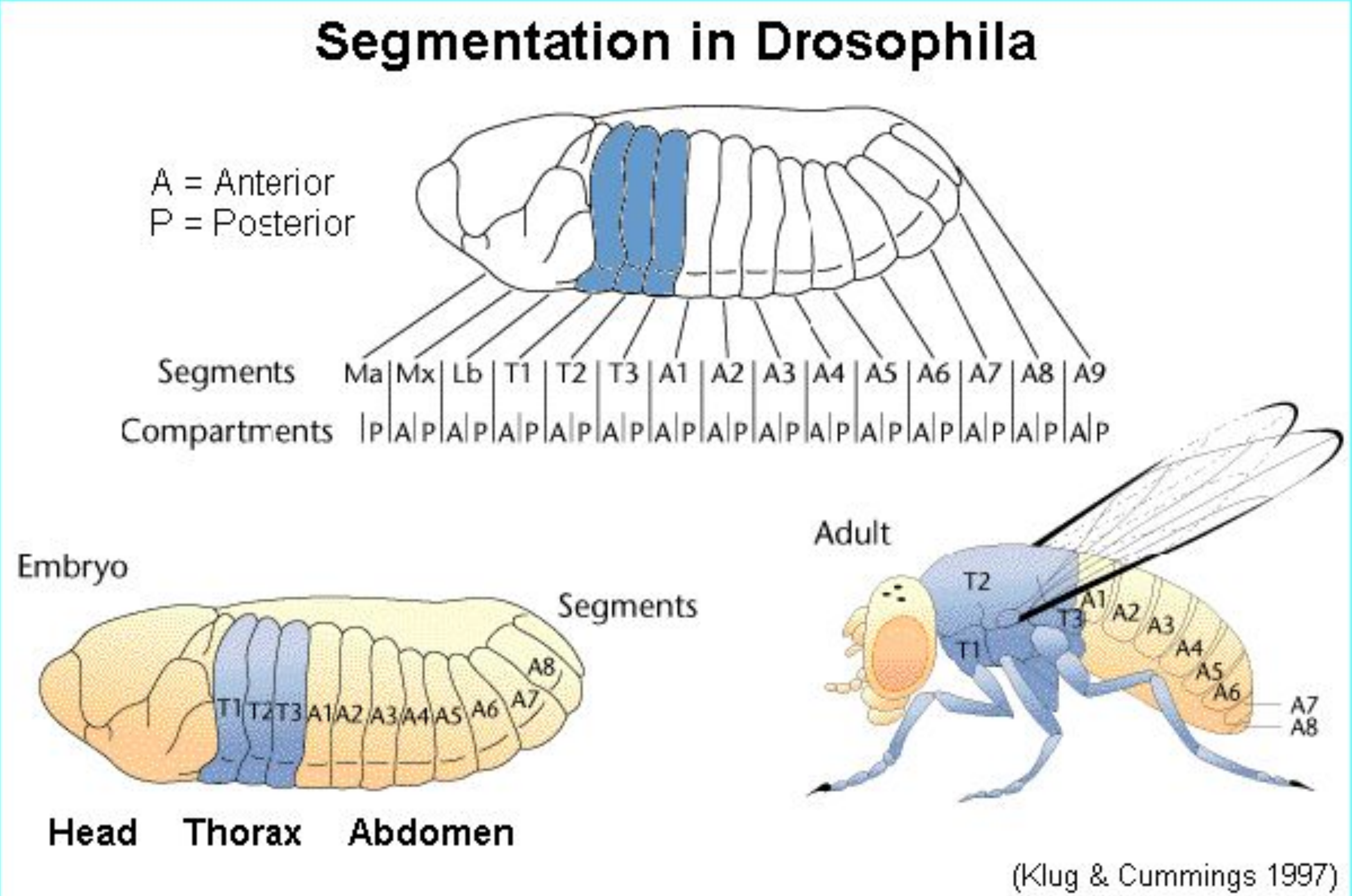
After fertilization, a series of steps results in selected transport of **Dorsal** into ventral nuclei, thus forming a D/V gradient

Segmentation genes act sequentially to divide the embryo into segments

- Normal Drosophila embryo divided into 15 segments
  - 3 head, 3 thoracic and 9 abdominal
  - Each will give rise to unique morphological features in adult



Парасегменты (compartments) соответствуют границам экспрессии различных генов сегментации и смещены влево от морфологически выраженных сегментов (на пол сегмента)



Production of Body Plan

The body plan is produced by sequential activation of three classes of segmentation genes

Segmentation Is Initiated by Localized RNAs at the Anterior and Posterior Poles of the Unfertilized Egg

1. Gap genes

-Map out the coarsest subdivision along the A/P axis

-All 9 genes encode transcription factors that activate the next gene class

2. Pair-rule genes

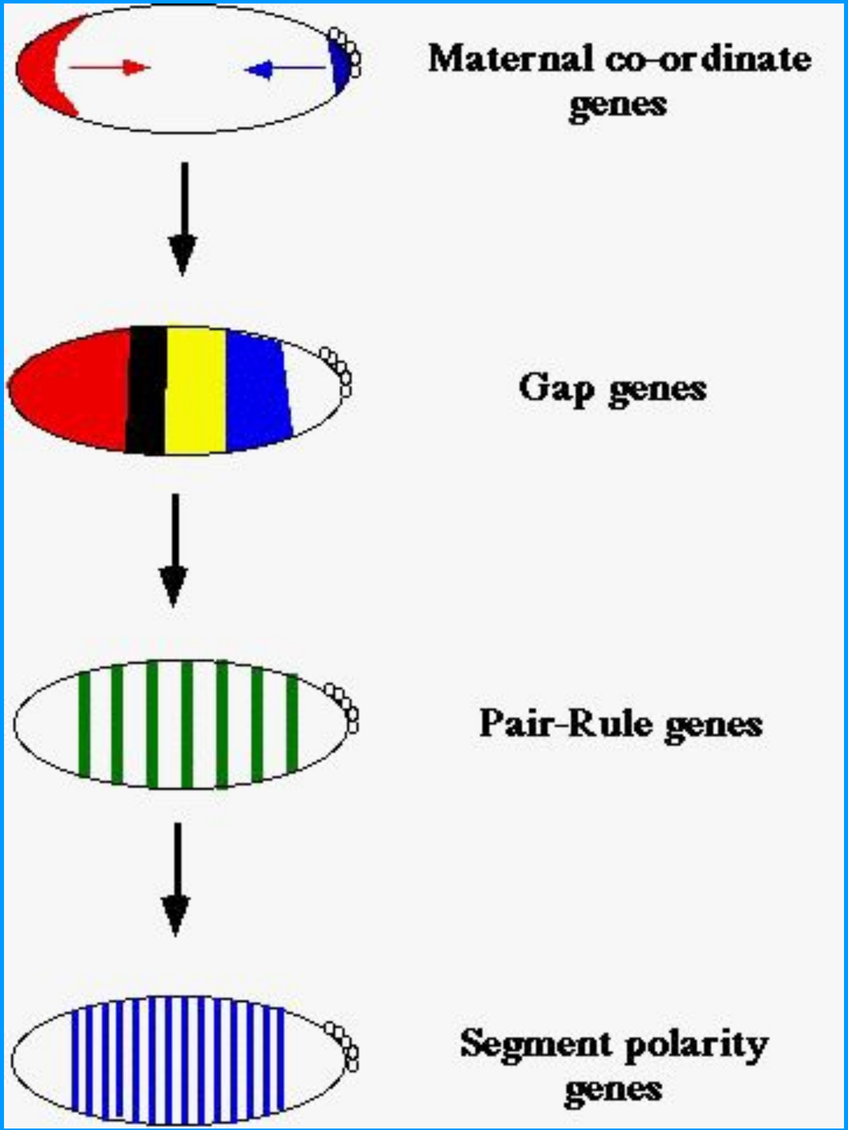
-Divide the embryo into seven zones

-The 8 or more genes encode transcription factors that regulate each other, and activate the next gene class

3. Segment polarity genes

-Finish defining the embryonic segments

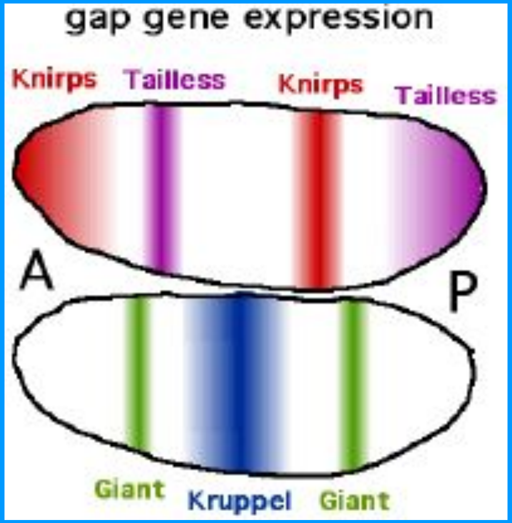
3 группы генов сегментации



- *Gap-гены,*
- гены *pair-rule*
- гены сегментной полярности.

- Gap genes
  - Expressed first
  - Gap mutants show a gap in segmentation pattern at positions where particular gene is absent
  - Binding sites in promoter have different affinities for maternal transcription factors
  - Gap genes encode transcription factors that influence expression of other gap genes

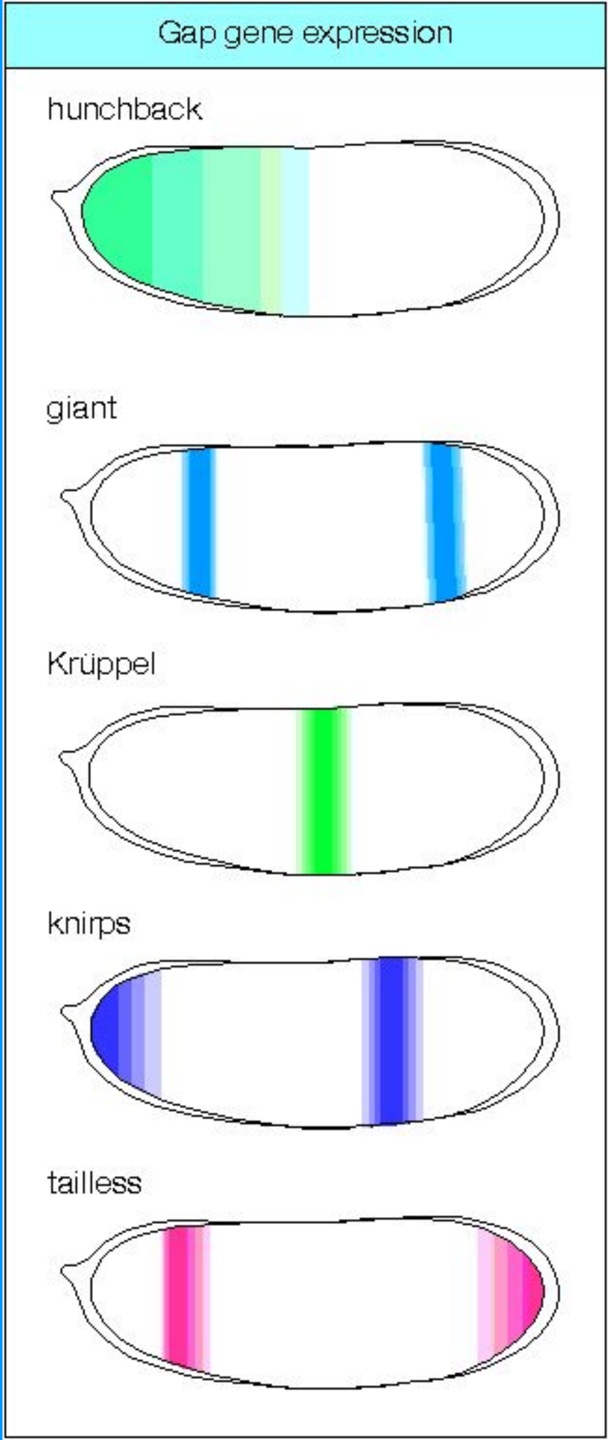
Gap genes



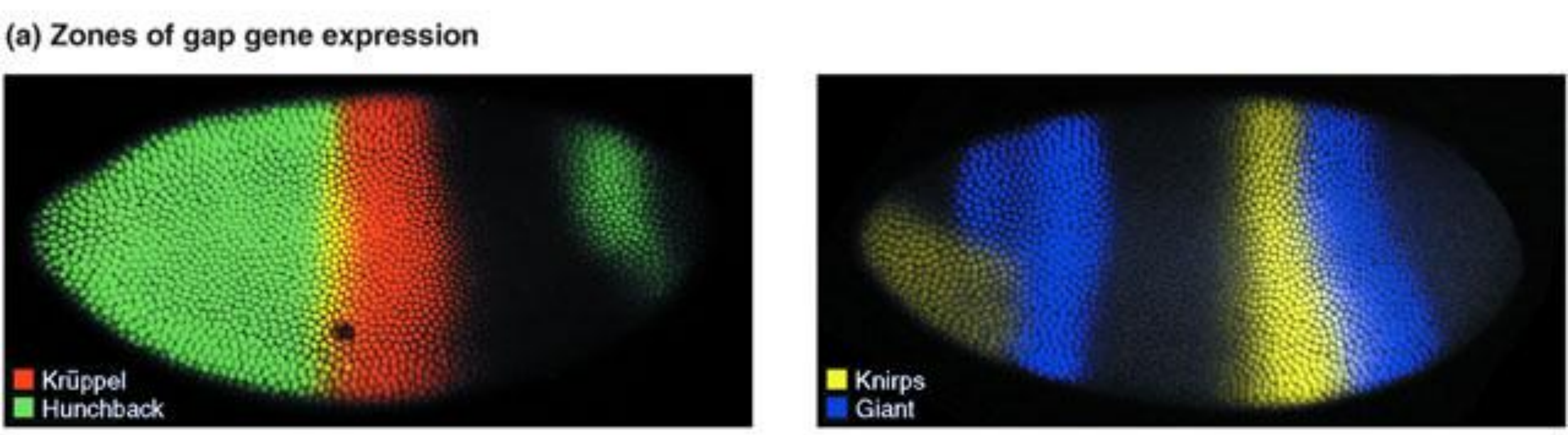
- *Hunchback, krüppel, giant, tailless* **and** ***knirps*****.**
- **Their expression patterns in the early embryo are determined by the maternal effect gene products**
- **These genes establish the segmented body plan of the embryo along the anterior-posterior axis.**

The A-P axis is divided into broad regions by gap gene expression

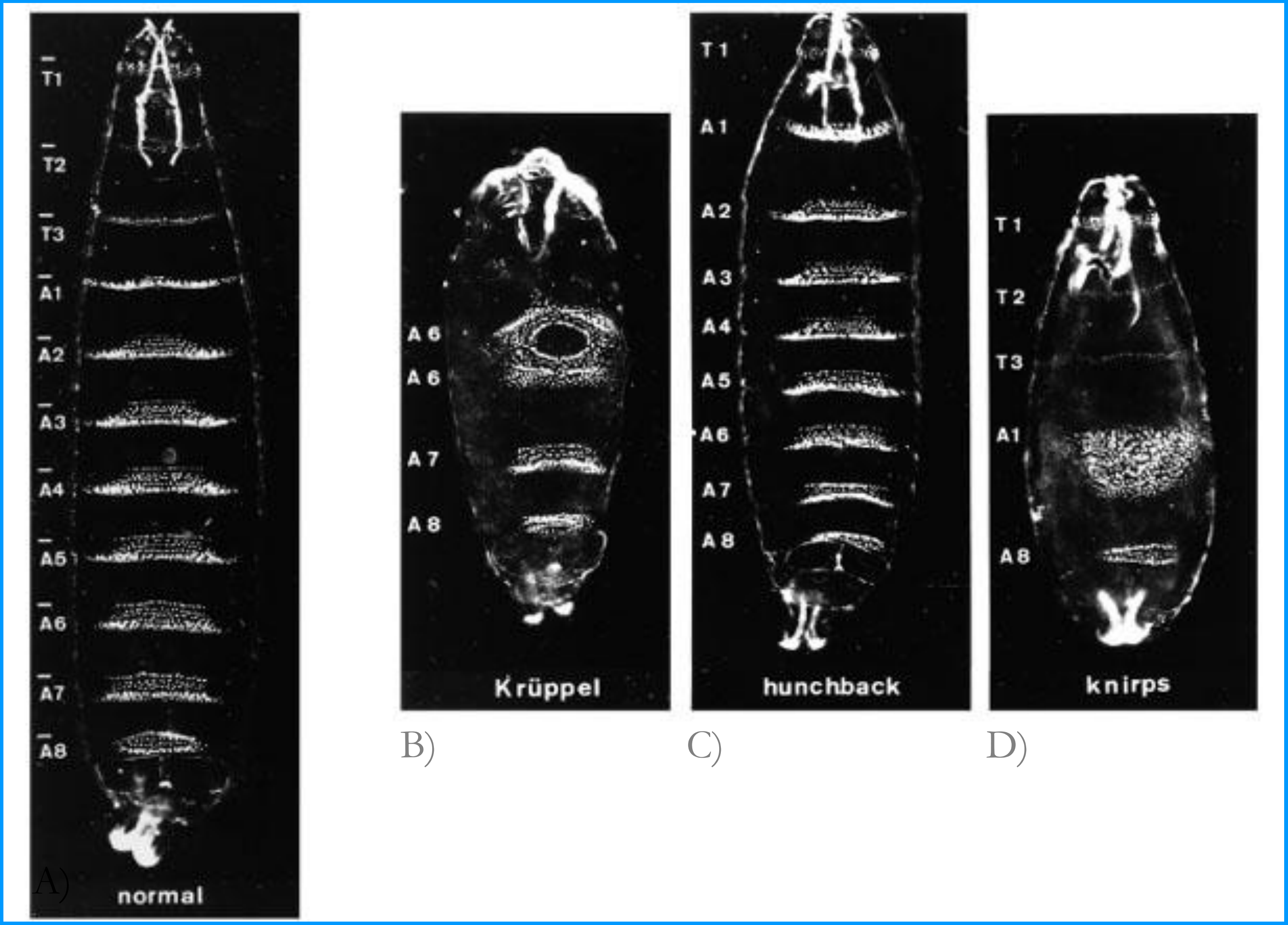
- **The first *zygotic* genes**
- Respond to maternally-derived instructions
- Short-lived proteins,
- gives bell-shaped distribution from source



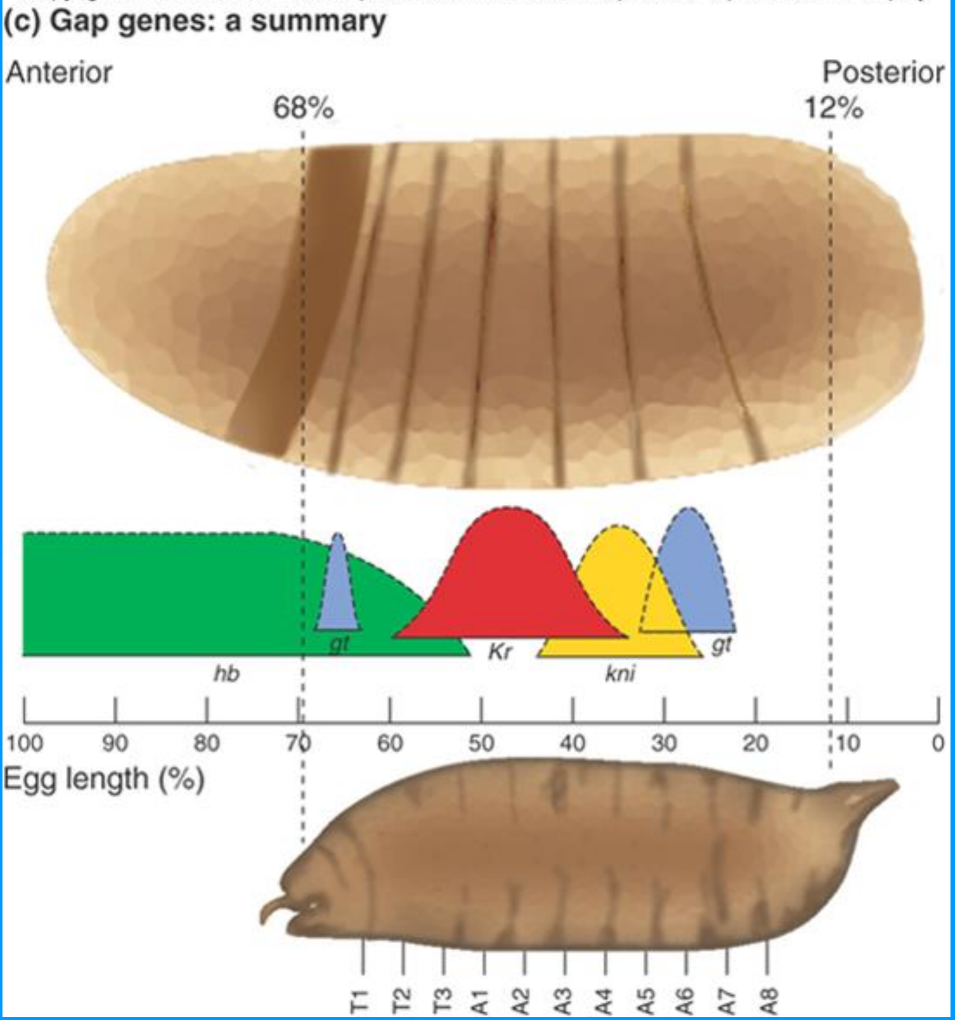
Zones of Expression of Four Gap Genes: *Hunchback*, *Kruppel*, *Knirps*, and *Giant* in Late Syncytial Blastoderm Embryos



Defects in Segmentation from Mutations in Gap Genes



Mutations in Gap Gene Result in Loss of Segments Corresponding to Zone of Expression

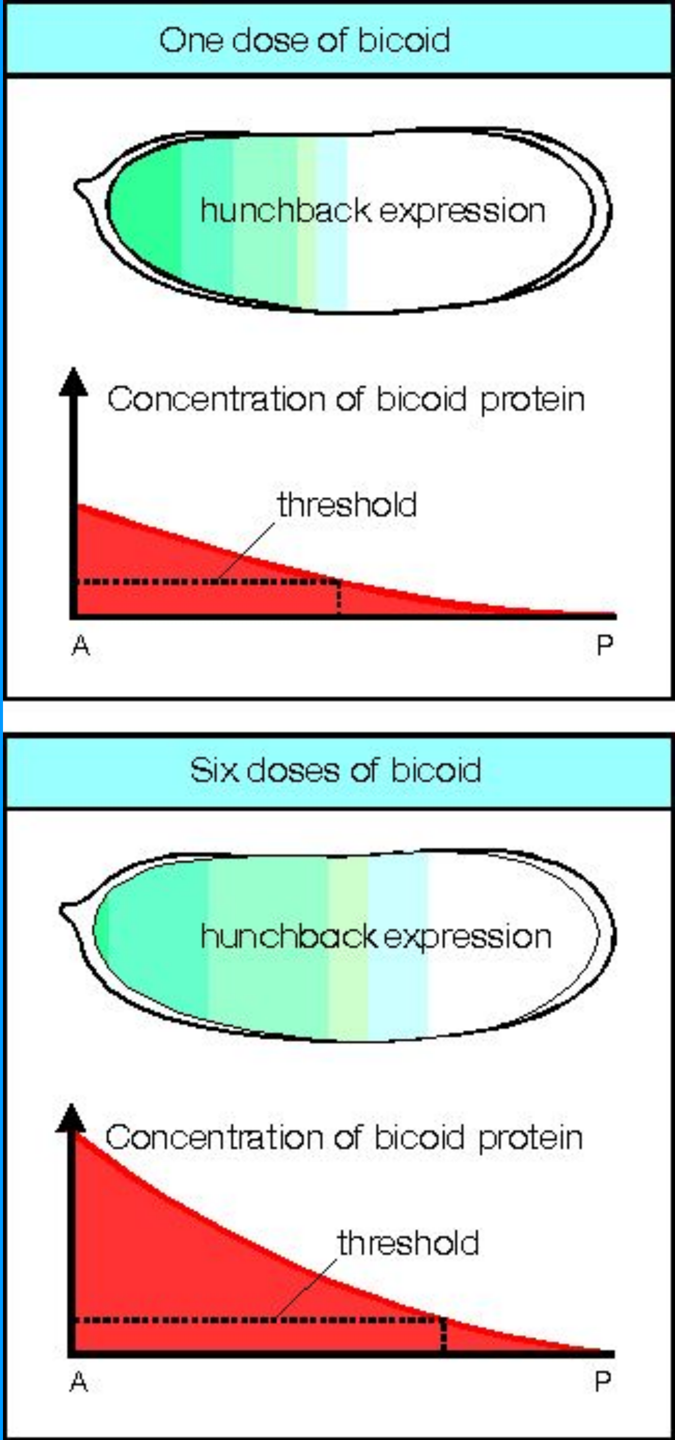


Transcription factors in cascade
GAP genes

- ***Hunchback (hb)*** **, a gap gene, responds to the dose of bicoid protein**

- **A concentration above threshold of bicoid activates the expression of *hb***

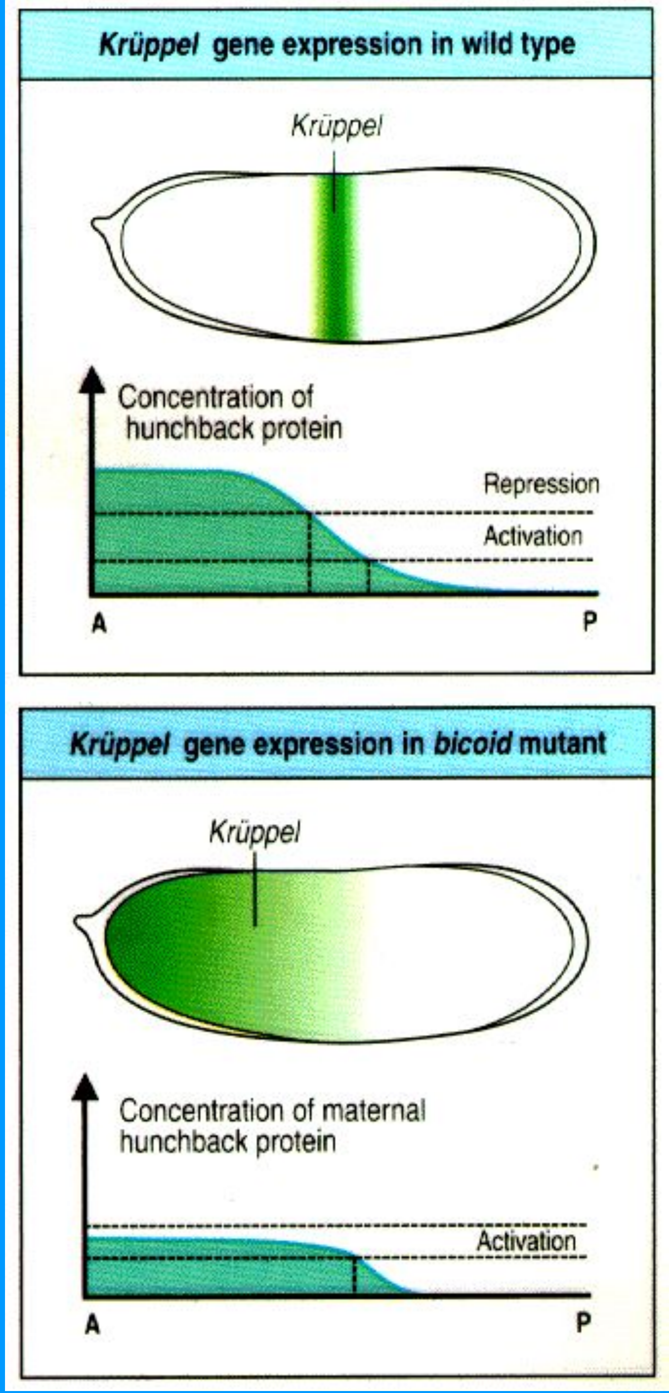
- **The more *bicoid* transcripts, the further back *hb* expression goes**

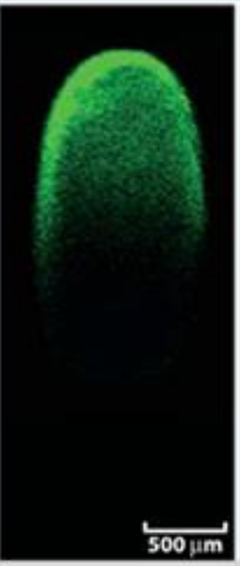


Krüppel reads two values

GAP genes

- *Krüppel (Kr)*, a gap gene, responds to the dose of hb protein
- A concentration **above minimum threshold** of hb activates the expression of *Kr*
- A concentration **above maximum threshold** of hb inactivates the expression of *Kr*



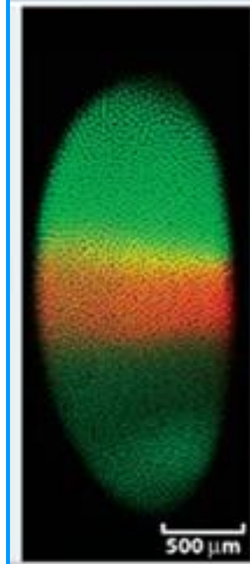


Establishing the Polarity of the Embryo

Fertilization of the egg triggers the production of bicoid protein from maternal RNA in the egg. The bicoid protein diffuses through the egg, forming a gradient. This gradient determines the polarity of the embryo, with the head and thorax developing in the zone of high concentration (*green* fluorescent dye in antibodies that bind bicoid protein allows visualization of the gradient).

Setting the Stage for Segmentation

About 21/2 hours after fertilization, bicoid protein turns on a series of brief signals from so-called gap genes. The gap proteins act to divide the embryo into large blocks. In this photo, fluorescent dyes in antibodies that bind to the gap proteins Krüppel (*orange*) and Hunchback (*green*) make the blocks visible; the region of overlap is yellow.

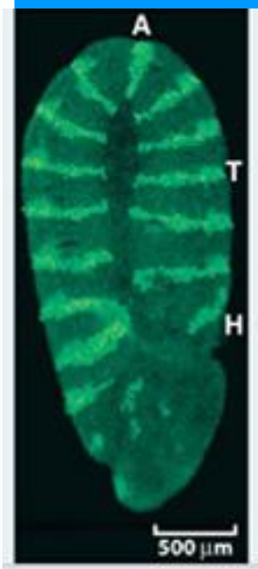


Laying Down the Fundamental Regions

About 0.5 hr later, the gap genes switch on the “pair-rule” genes, which are each expressed in seven stripes. This is shown for the pair-rule gene *hairy* . Some pair-rule genes are only required for even-numbered segments while others are only required for odd numbered segments.

Forming the Segments

The final stage of segmentation occurs when a “segment-polarity” gene called engrailed divides each of the seven regions into halves, producing 14 narrow compartments. Each compartment corresponds to one segment of the future body. There are three head segments (H, *bottom right*), three thoracic segments (T, *upper right*), and eight abdominal segments (A, *from top right to bottom left*).



We know some misterious mutations, which generate horroristic monsters

Homeotic mutations

Homeotic-Selector/ HOX Genes

Production of Body Plan

Segment identity arises from the action of **homeotic genes**

-Mutations in them lead to the appearance of normal body parts in unusual places

-*Ultrabithorax* mutants produce an extra pair of wings

Production of Body Plan

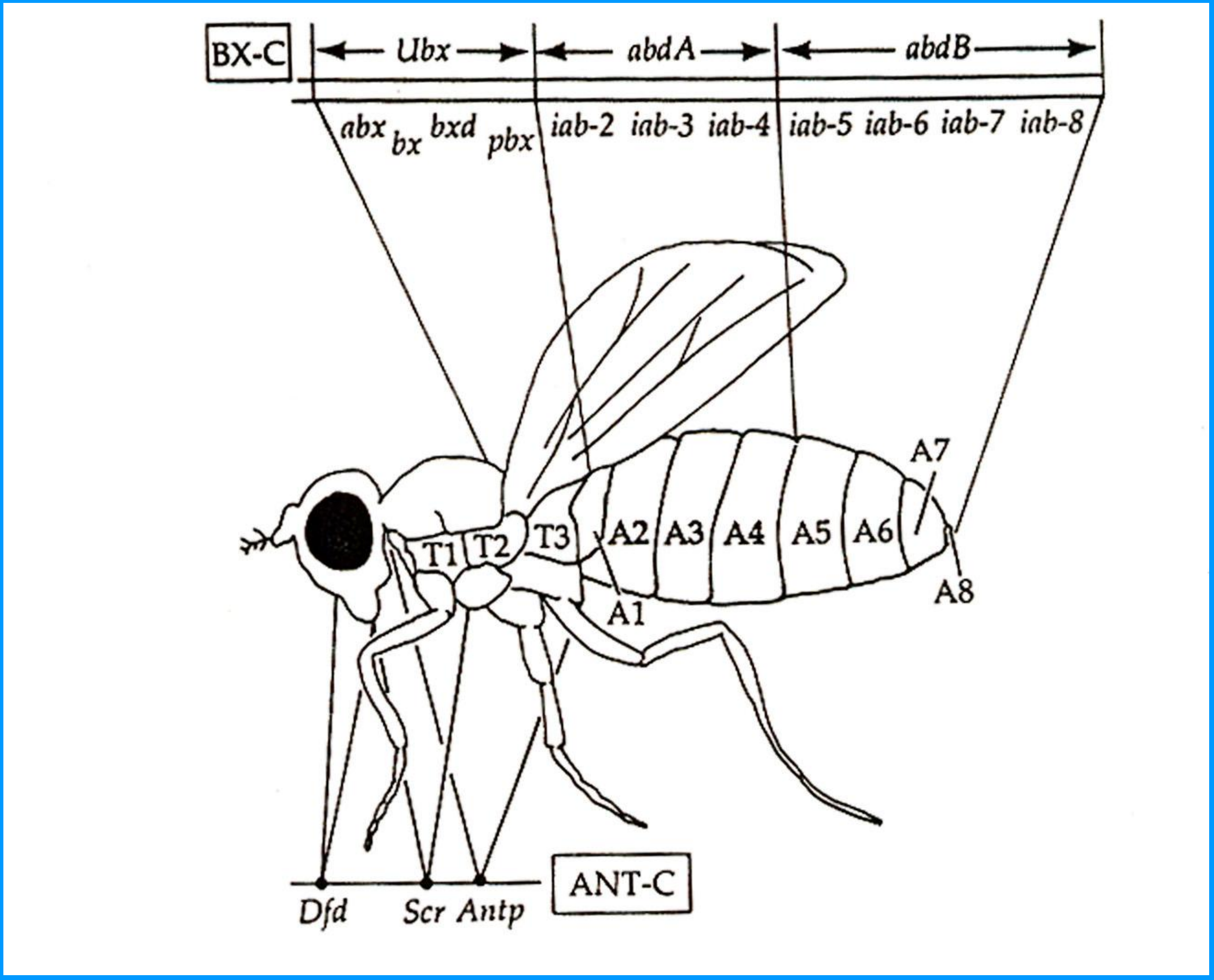
Homeotic gene complexes

-The **HOM complex** genes of *Drosophila* are grouped into two clusters

-**Antennapedia complex**, which governs the anterior end of the fly

-**Bithorax complex**, which governs the posterior end of the fly

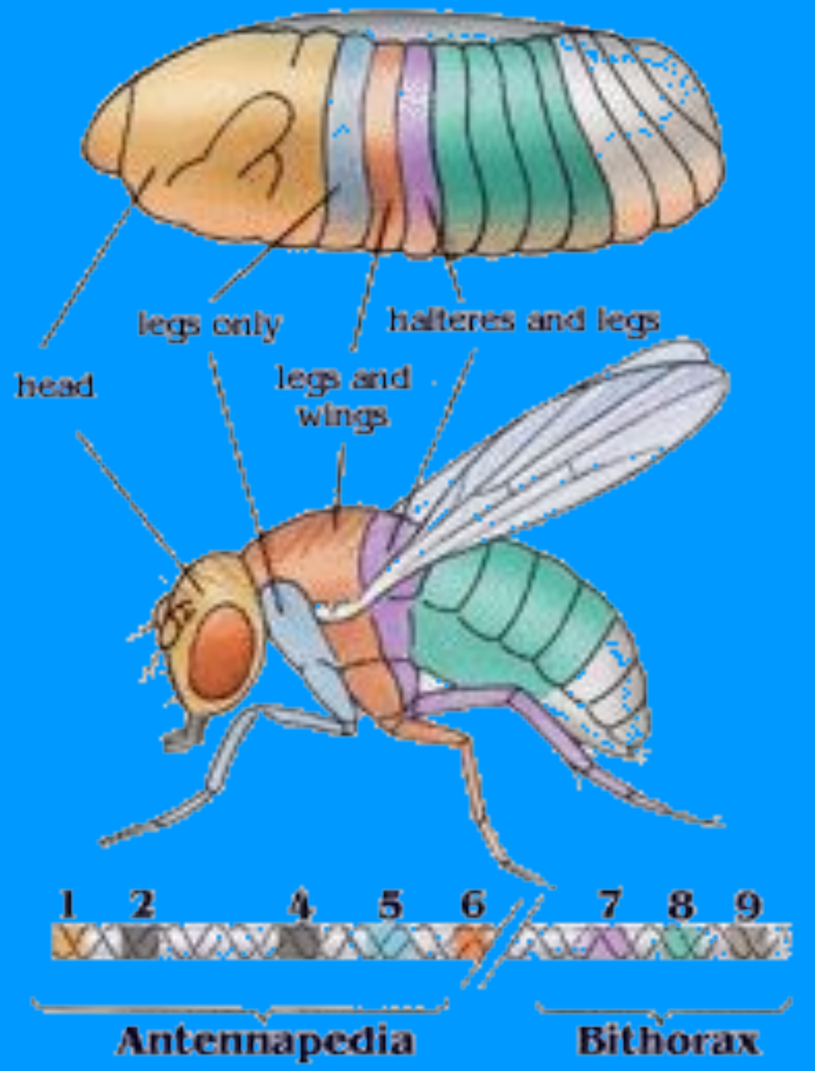
-***Interestingly, the order of genes mirrors the order of the body parts they control***



Homeotic genes specify body segment identity in *Drosophila*.

Fig. 14

Homeotic Genes Determine Specialization of Segments



Гомеозисные гены

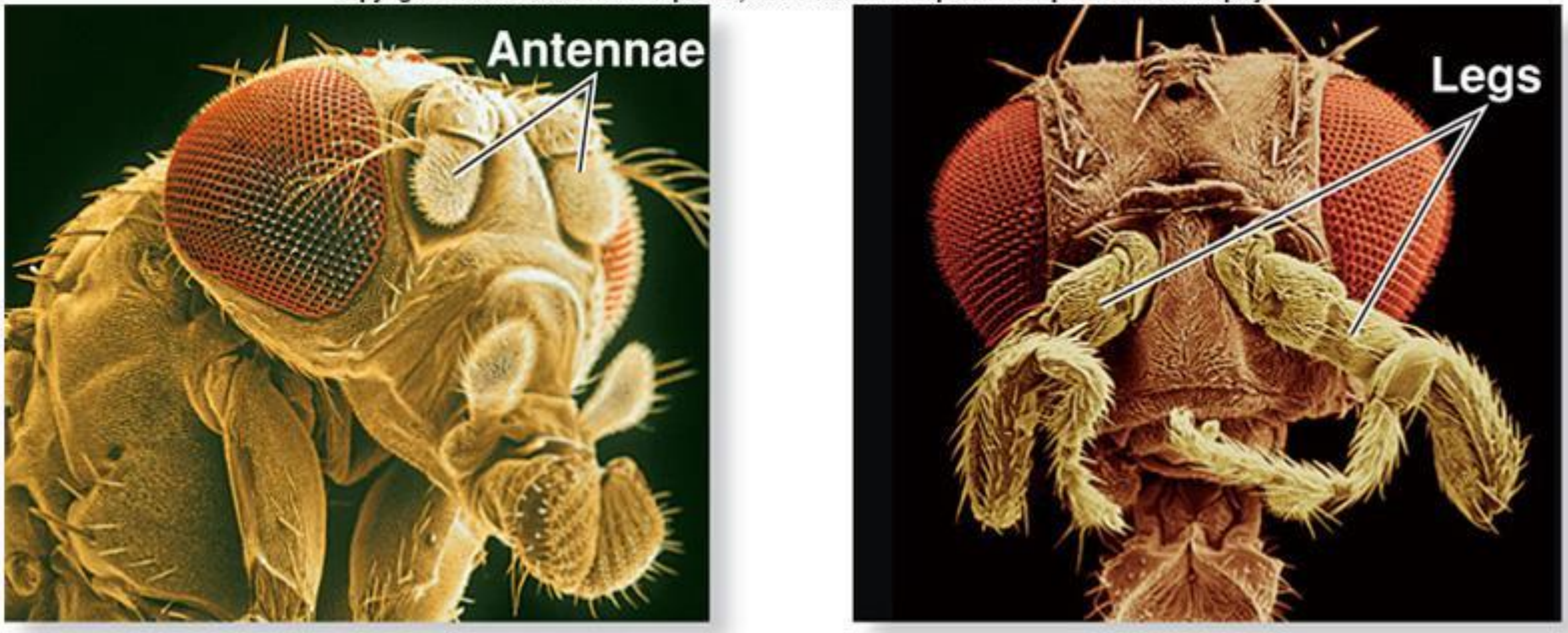
- Большой класс генов
- контролируют развитие части тела
- из определенного сегмента.

- В результате гомеозисной мутации
- из данного сегмента развивается
- другая часть тела

Наиболее известны

- *Antennapedia-complex (Ant-C).*
- *Bithorax-complex (BX-C)* **и**

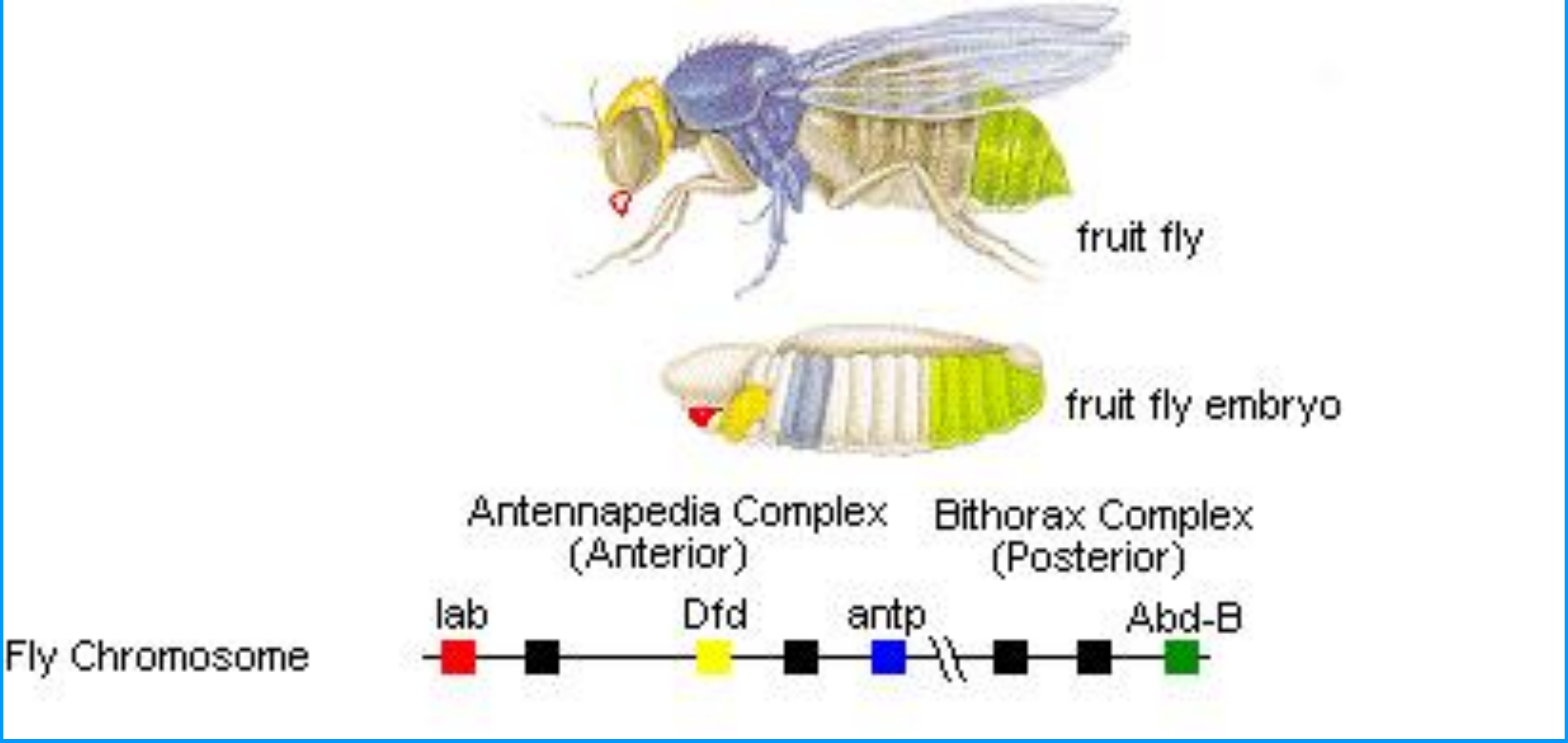
- ***Antennapedia complex***
- 5 genes that affect the anterior part of the fly
- When mutated, legs grow instead of antennae



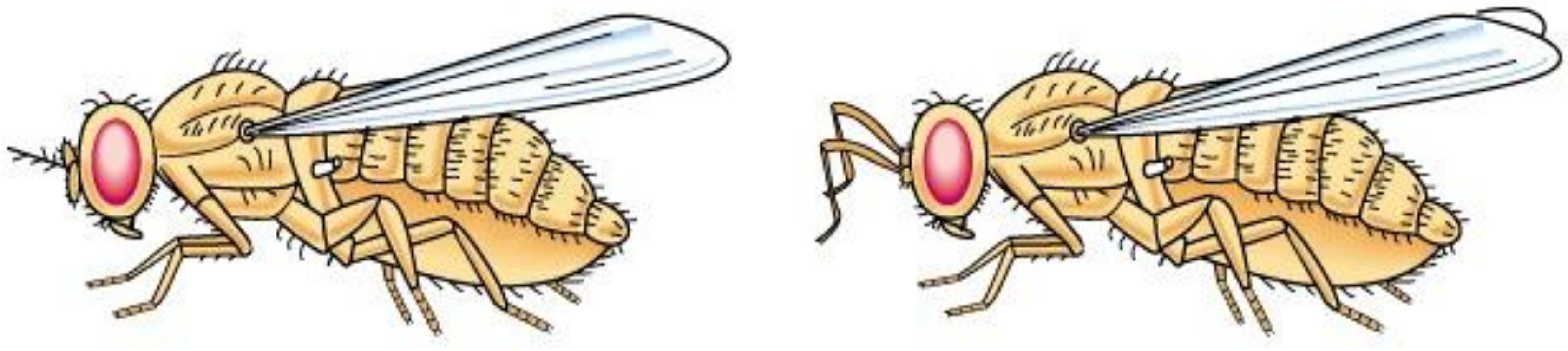
(a) Normal fly (b) Mutant fly

a: © Juergen Berger/Photo Researchers, Inc.; b: F. R. Turner, Indiana University

Homeotic Genes: Master Regulatory Genes

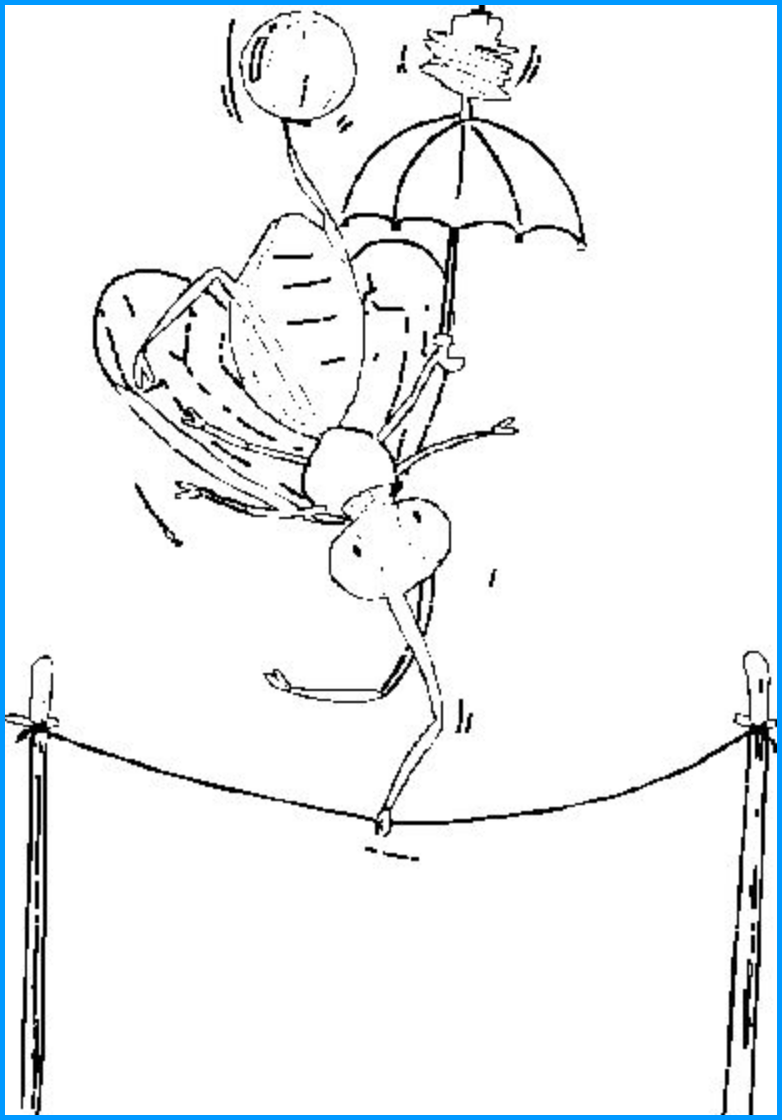


Homeotic mutation: Master Regulatory Genes



Normal fly

Antennapedia mutant fly

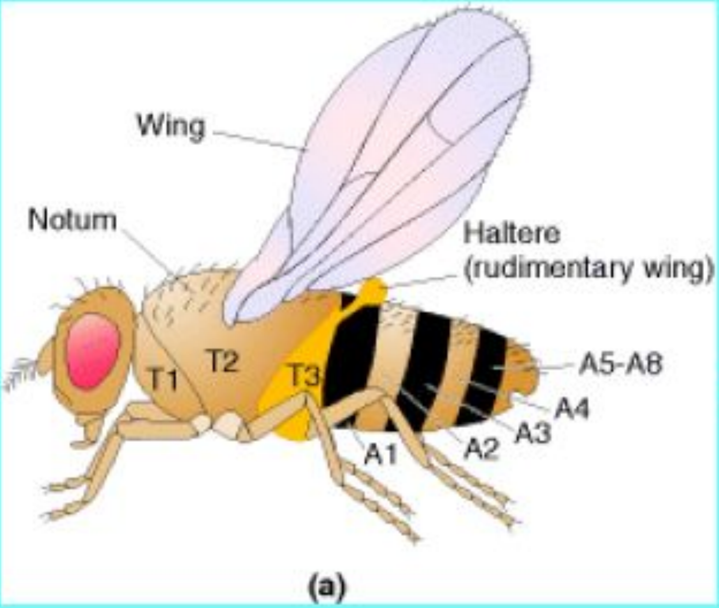


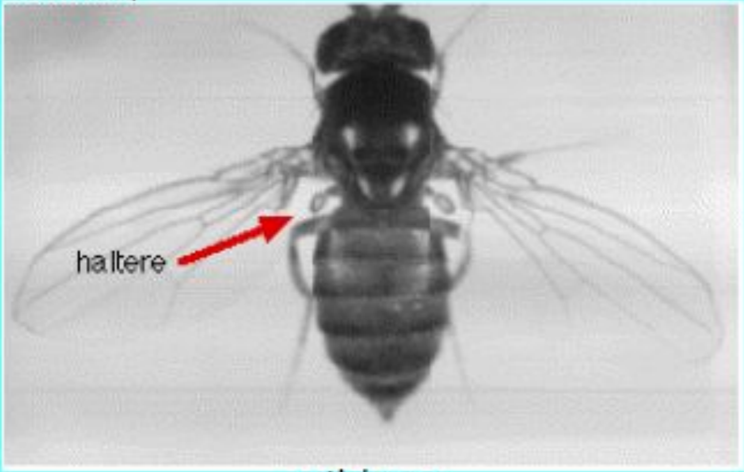
Halteres into wings

One structure is placed in the position where normally another structure would be (e.g. a pair of wings instead of halteres)

Gilbert, SF (2003) Developmental Biology, 7th ed.

Гомейозисная мутация у *Drosophila (*подавление активности гена *ultrabithorax)* приводит к образованию второй пары крыльев вместо жужжалец





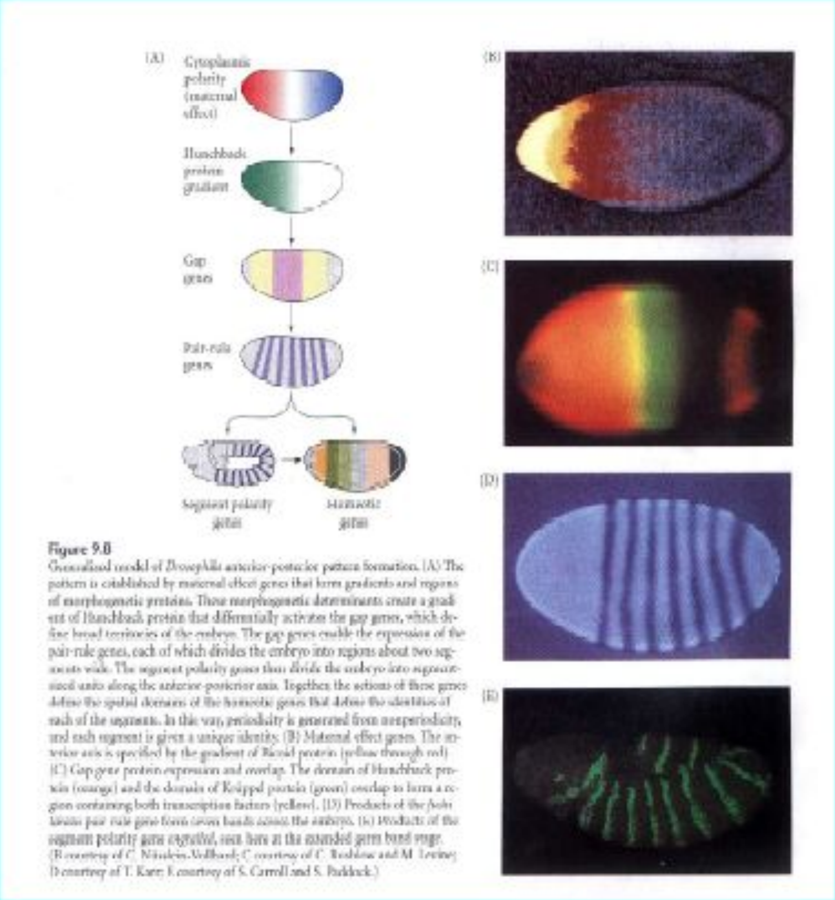
wildtype

Ultrabithorax

Гомеозисные гены относятся к селекторным генам. Через свои белковые продукты -транскрипционные факторы - они избирательно воздействуют на гены начальных звеньев генетических формообразовательных программ, определяя выбор дифференцировки целого участка тела организма. Кодируемые ими транскрипционные факторы для избирательности воздействия могут использовать кофакторы. У Drosophila - это белковые продукты генов *Extradenticle, tearshirt* и др.

Гомейозисные (гомеотические) гены

Активности гомейозисных генов инициируются продуктами экспрессии генов сегментации, а затем поддерживаются самостоятельно



Production of Body Plan

Homeotic gene complexes

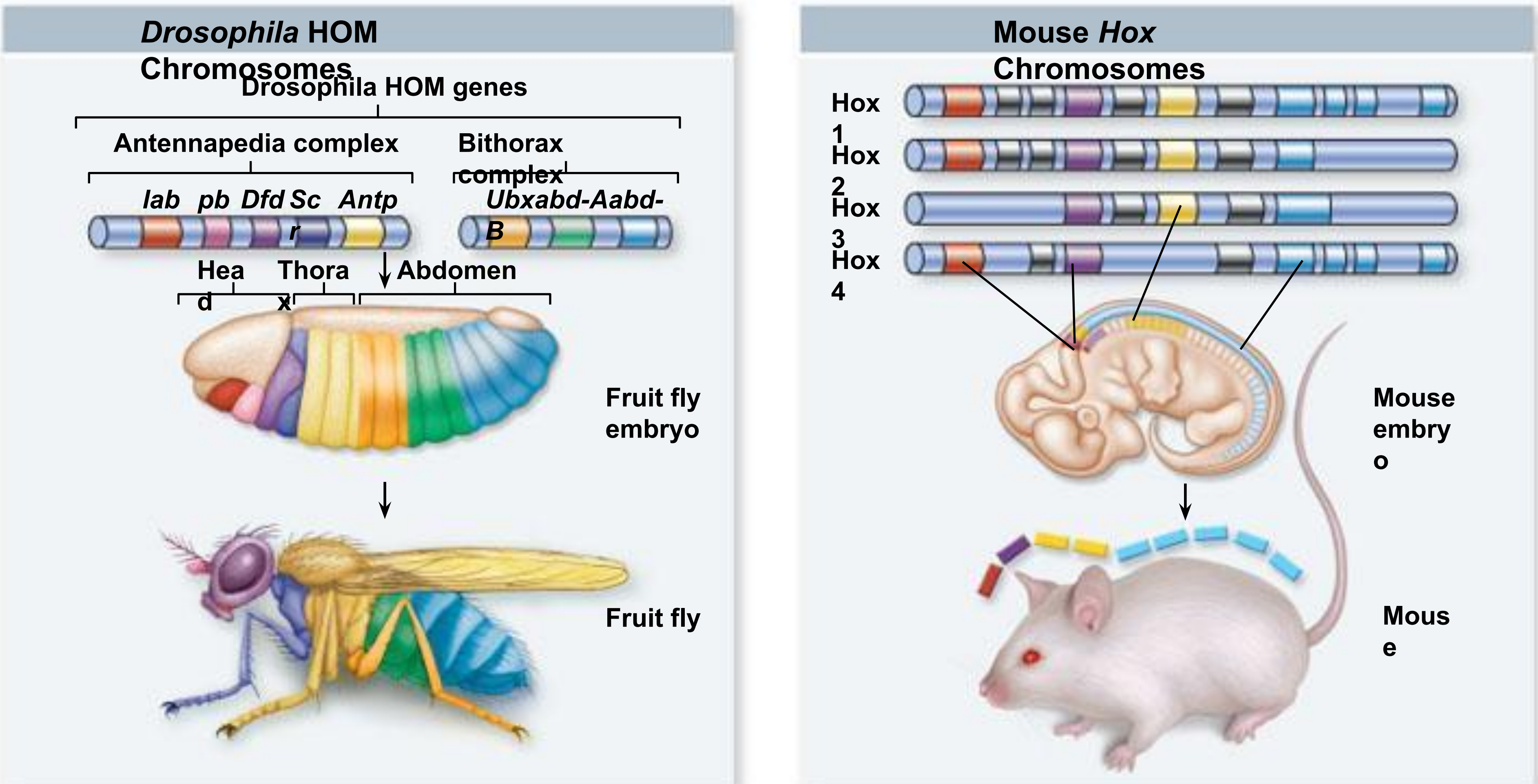
-All of these genes contain a conserved 180-base sequence, the **homeobox**

-Encodes a 60-amino acid DNA-binding domain, the **homeodomain**

-Homeobox-containing genes are termed ***Hox* genes**

-Vertebrates have 4 *Hox* gene clusters

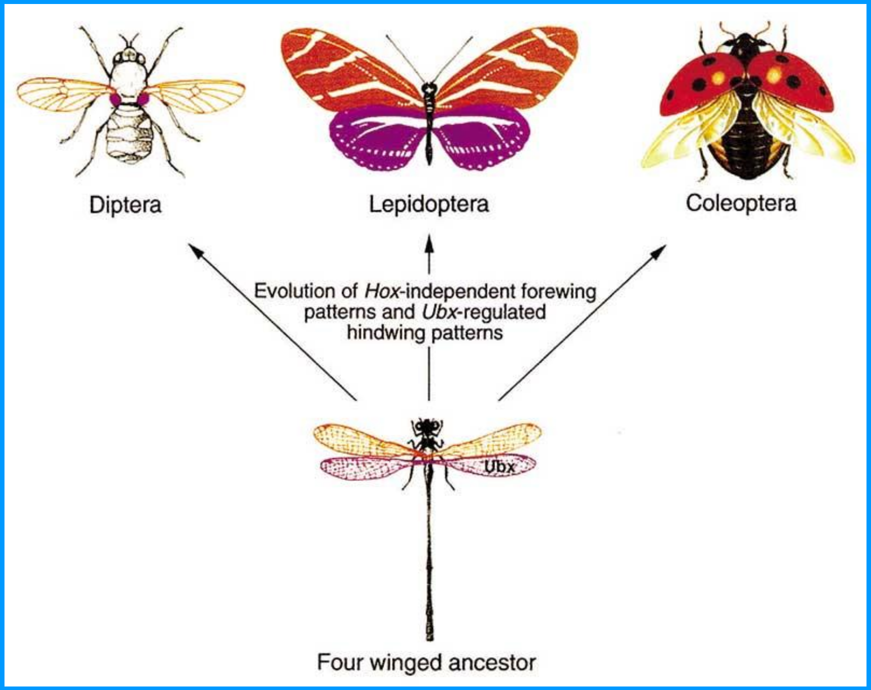
Production of Body Plan



a.

b.

Как произошли мухи?



Мухи эволюционировали из насекомых, имевших 4 крыла, Насекомые произошли из членистоногих, имевших множество ног.
В ходе эволюции у мух сформировались группы генов:
а) подавляющие развитие ног на брюшных сегментах многоножкоподобных предков,
б) подавляющих развитие второй пары крыльев,
В) формирующие новые структруры: гальтеры и брюшные сегменты.

К чему приводит делеция *BX-C*?

- Эмбрион развивается до определенной стадии и затем гибнет

- Эмбрион имел
- проторакальный сегмент, а
- все остальные — мезоторакальные

- Если бы этот организм выжил до взрослой мухи,
- она бы имела 10 пар крыльев и 10 пар ног

- Функция гена *BX-C*
- - инактивация генов, формирующих ноги и крылья во всех последующих после второго торакального сегментах,
- - развитии всех структур на брюшных сегментах

Предполагаемая макроэволюционная роль гомейозисных мутаций - вклад биологии развития в эволюционную теорию. Однократная мутация одного или группы Hom-C генов может привести к образованию (утрате) пары крыльев или ножек

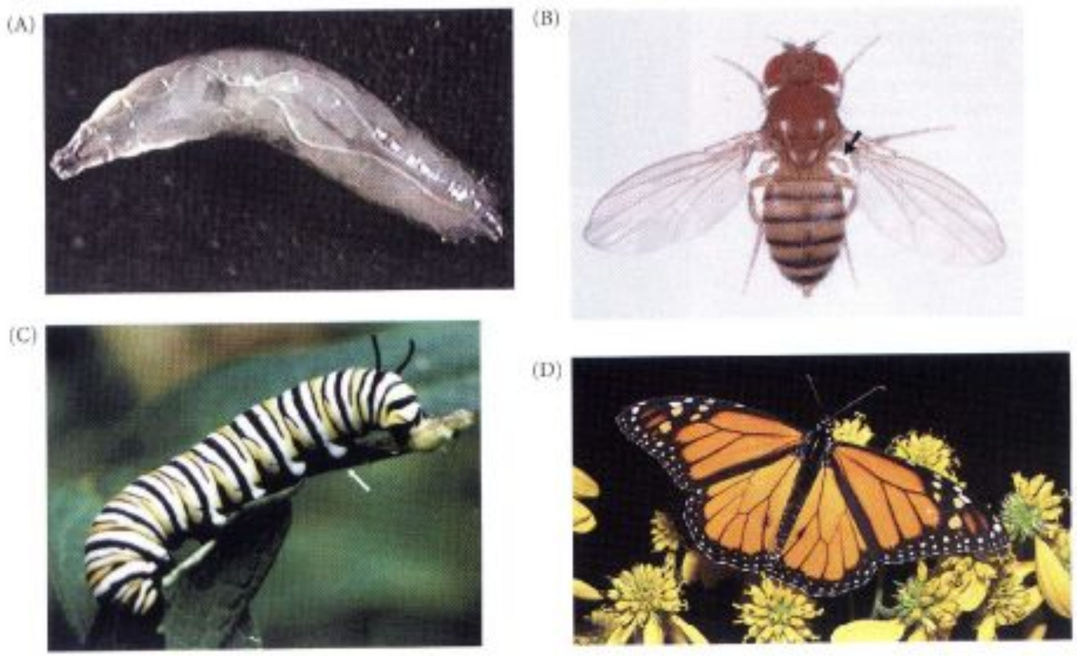
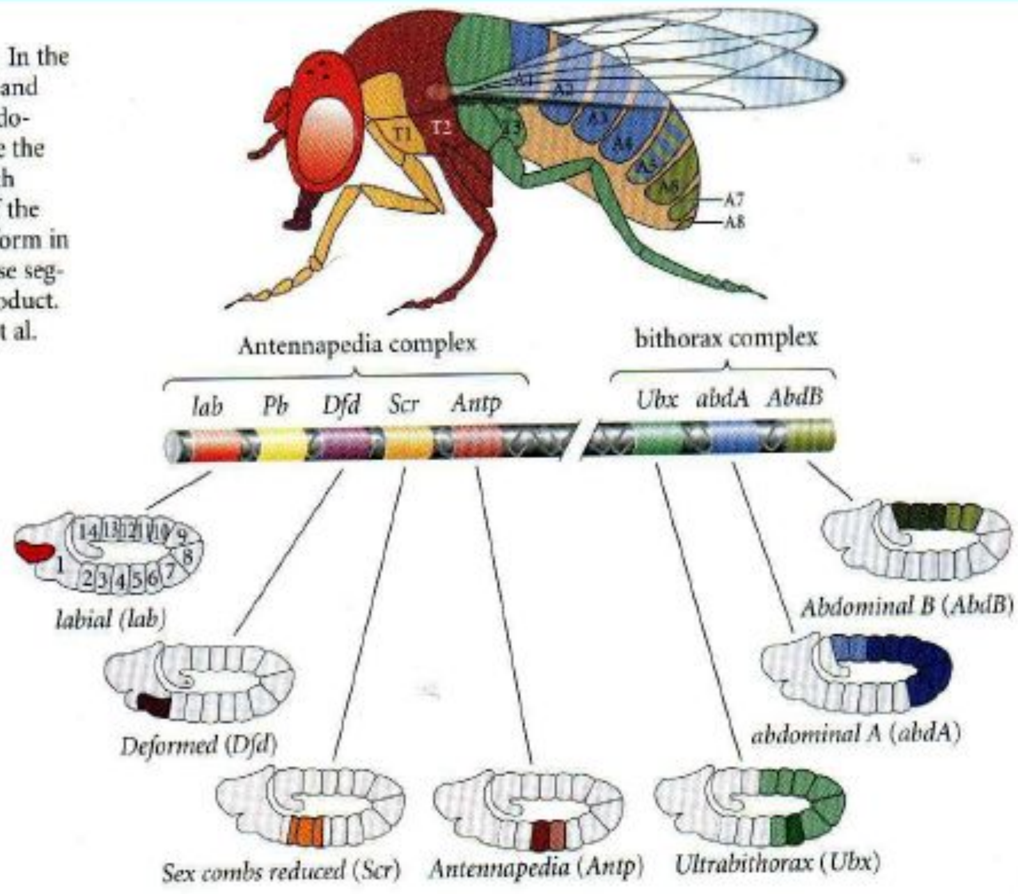


Figure 22.4
Differences in larval and adult morphology due to Hox gene differences. (A, B) Larva and adult of *Drosophila*, a dipteran. An arrow points to one of the halteres of the adult. The larva lacks prolegs; its anterior end is at the left. (C, D) Larva and adult of *Danaus plexippus*, the monarch butterfly, a lepidopteran. The anterior of the caterpillar is to the left, and a proleg is indicated by an arrow. The adult has hindwings rather than halteres. The regulation of Hox genes determines the presence of prolegs, and the targets of Hox genes determine whether the third thoracic segment is to generate halteres or hindwings. (A courtesy of M. Tyler; B courtesy of E. B. Lewis; C courtesy of G. Savage; D by Bill Beatty/Visuals Unlimited.)

Кластер гомейозисных генов Drosophila - Hom-C. Активность генов в зародыше и во взрослой особи. Последовательное расположение генов в кластере (на 3-й хромосоме) определяет последовательность их экспрессии в парасегментах вдоль передне-задней оси

Figure 9.27
Homeotic gene expression in *Drosophila*. In the center are the genes of the Antennapedia and bithorax complexes and their functional domains. Below and above the gene map are the regions of homeotic gene expression (both mRNA and protein) in the blastoderm of the *Drosophila* embryo and the regions that form in the adult fly. Darker shaded areas are those segments or parasegments with the most product. (After Dessain et al. 1992 and Kaufman et al. 1990.)



Vertebrates have four Hox complexes, with about 10 genes each.

They can be aligned in 13 groups of paralogues.

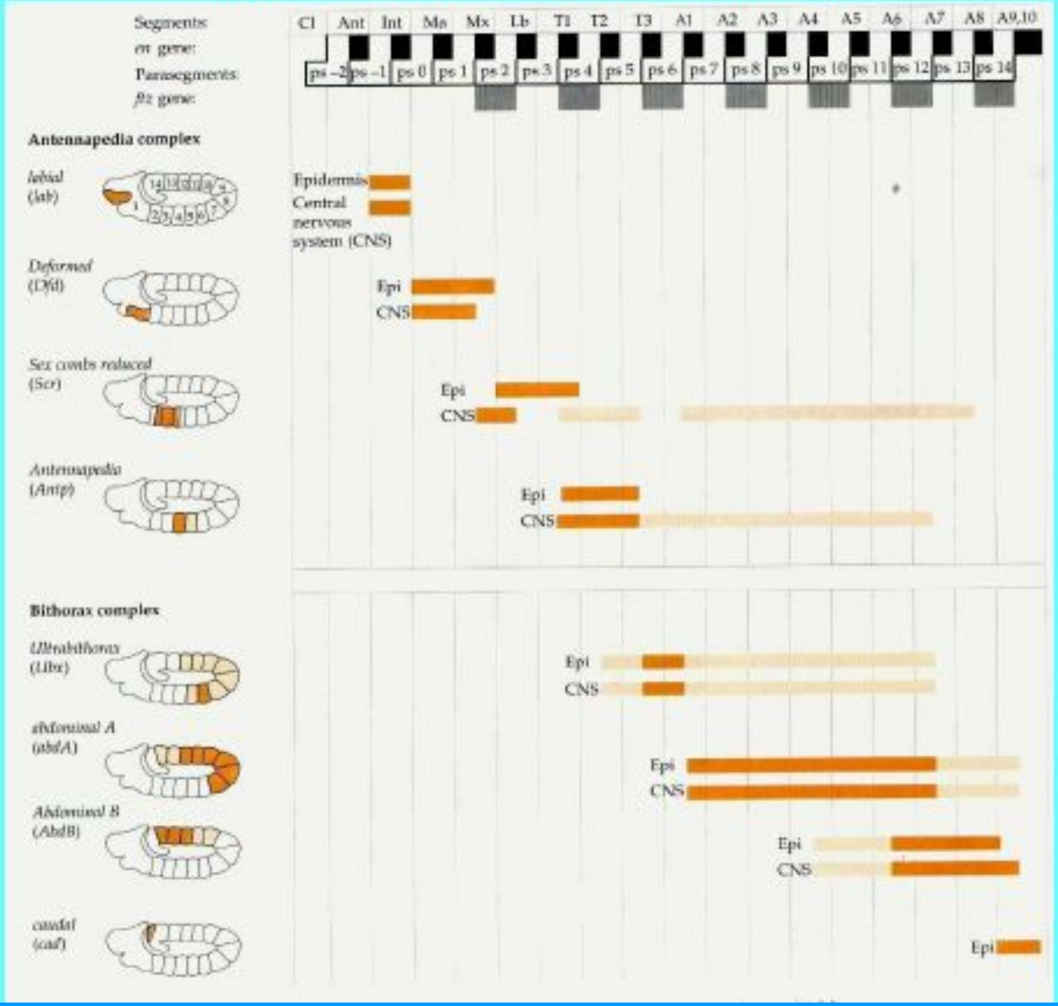
They display colinearity:

a) **Temporal colinearity: genes on one end of the complex are expressed first, those on the other (posterior) end are turned on last.**

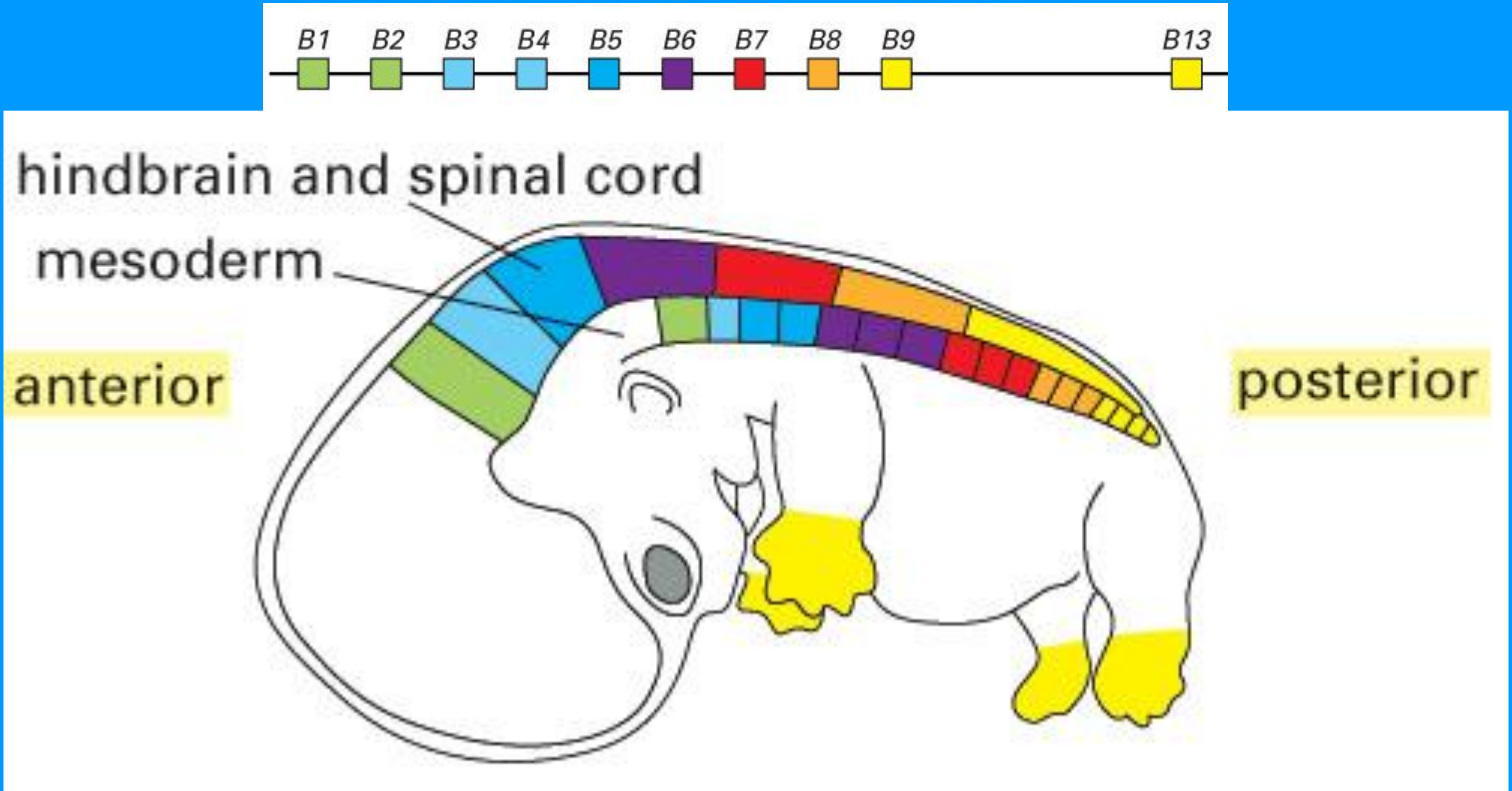
b) **Spatial colinearity: the more anteriorly expressed genes are in one end, the more posterior ones at the other end of the gene complex.**

c) **Anterior Hox genes are activated sequentially by retinoic acid.**

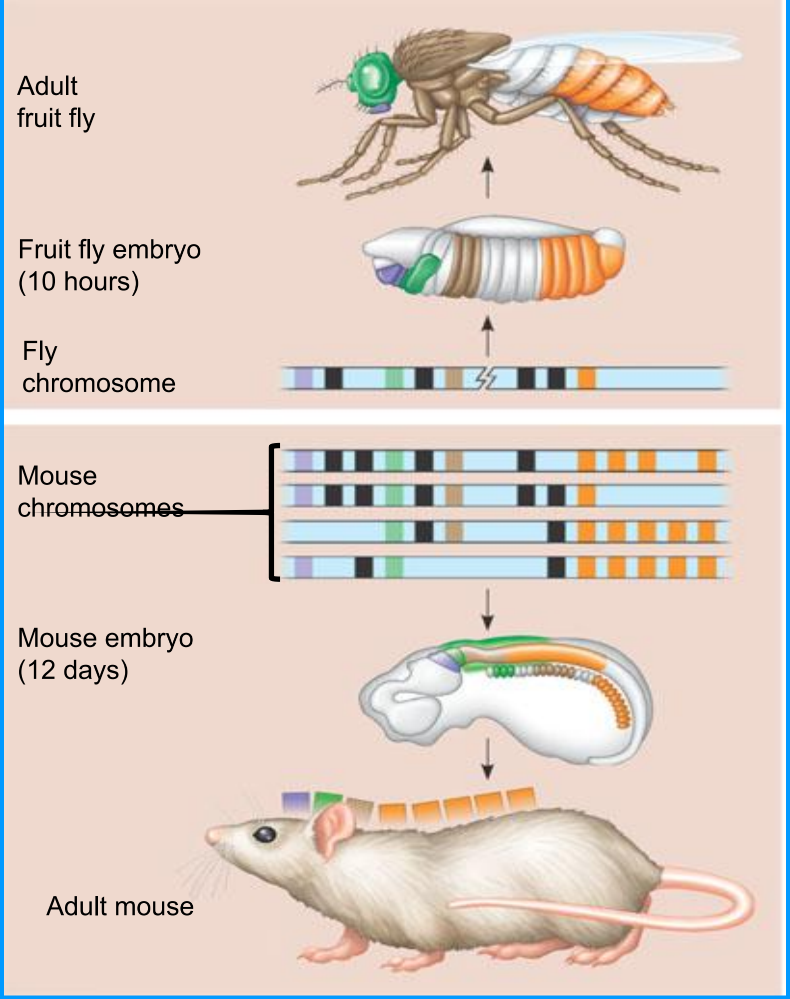
Районы экспрессии гомеотических генов в бластодерме зародыша Drosophila и несколькими часами позже в нервной системе (тёмные полосы - белок, светлые -иРНК)



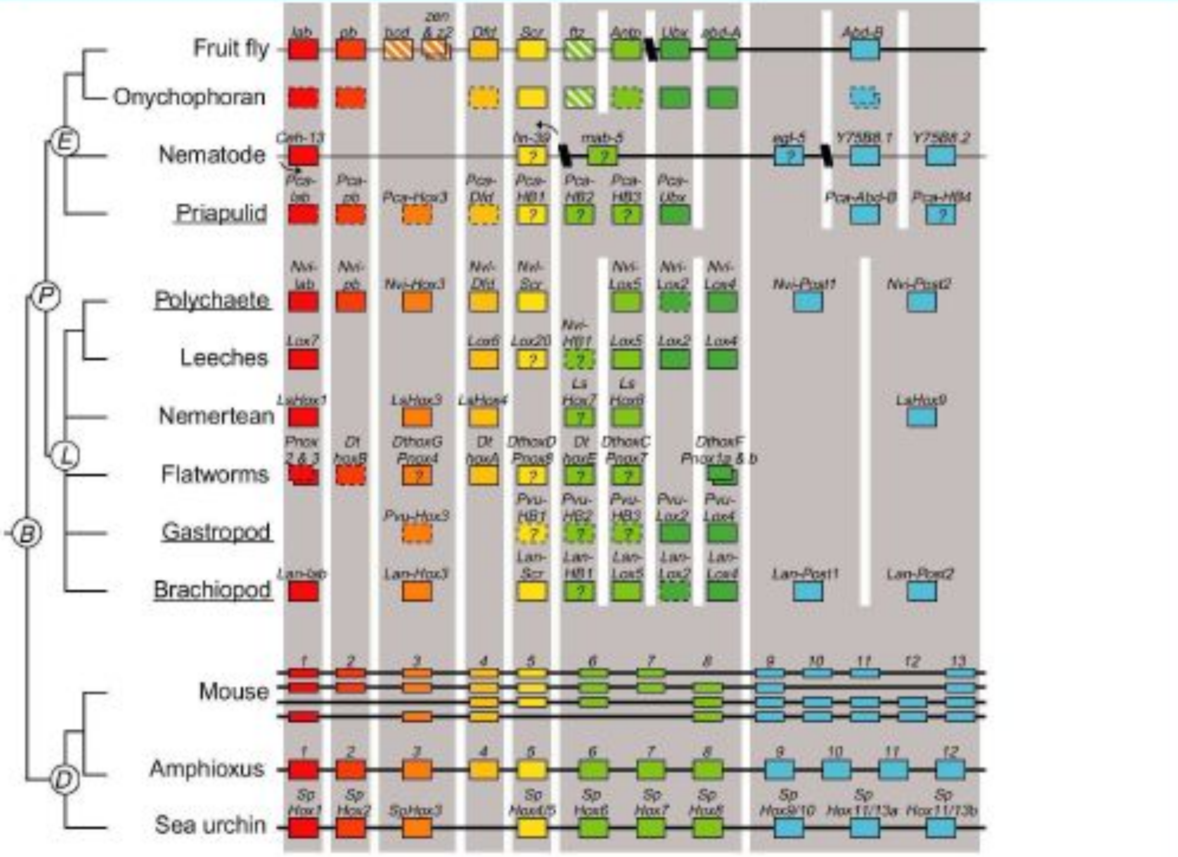
Temporal and spatial colinearity: order of Hox genes in DNA follows the antero-posterior body axis.



- **An identical or very similar nucleotide sequence**
  - **Has been discovered in the homeotic genes of both vertebrates and invertebrates**



Кластеры гомейозисных генов высоко консервативны. Они сформированы в явном виде у Bilateria и присутствуют в организмах всех типов животных, включая человека.



igure 3 Distribution of Hox genes in bilaterians. The tree on the left summarizes ne 18S rDNA phylogeny of Aguinaldo *et al.*[2]. B, common bilaterian ancestor; D, ommon deuterostome ancestor; E, stem ecdysozoan; L, stem lophotrochozoan; ; common protostome ancestor. Horizontal black lines indicate mapping data when available). Vertical white bars delineate orthologous genes or groups of genes. Uncertain orthology relationships are indicated by question marks, dashed boxes indicate short PCR fragments, solid boxes indicate complete or almost complete homeoboxes, and striped colours indicate fast-evolving arthropod Hox sequences. The organisms studied in this paper are underlined.

У позвоночных выявляется не менее четырех кластеров паралогичных гомейозисных генов. Число генов в кластерах выше, чем у Drosophila, но не все паралогичные гены сохраняются в отдельных кластерах

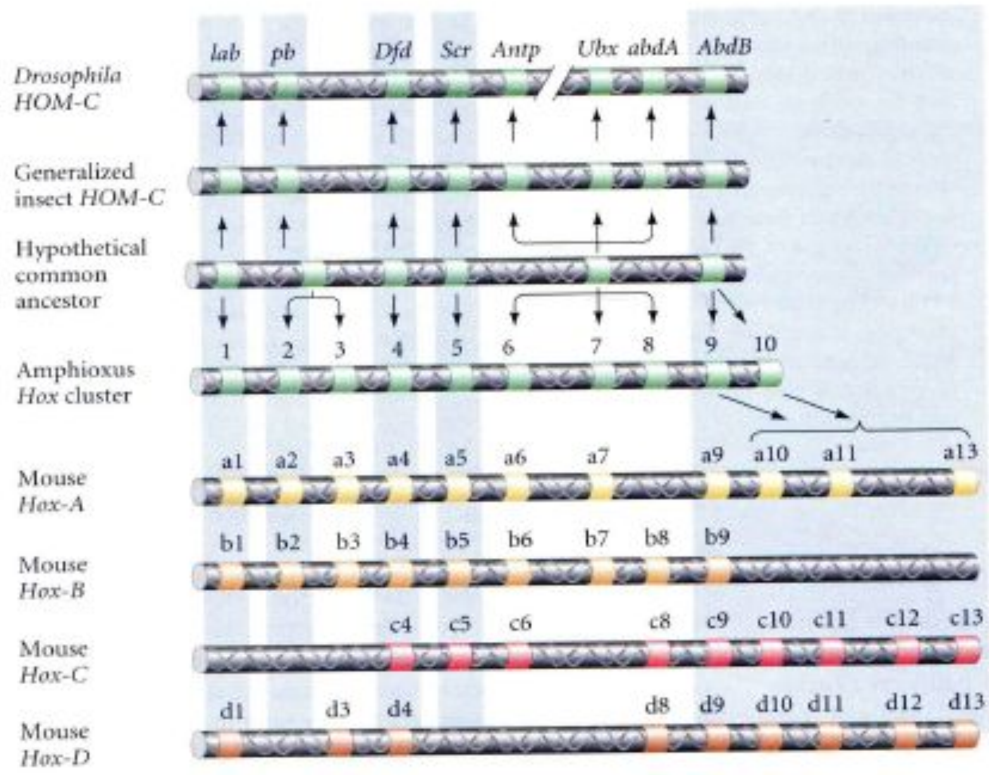
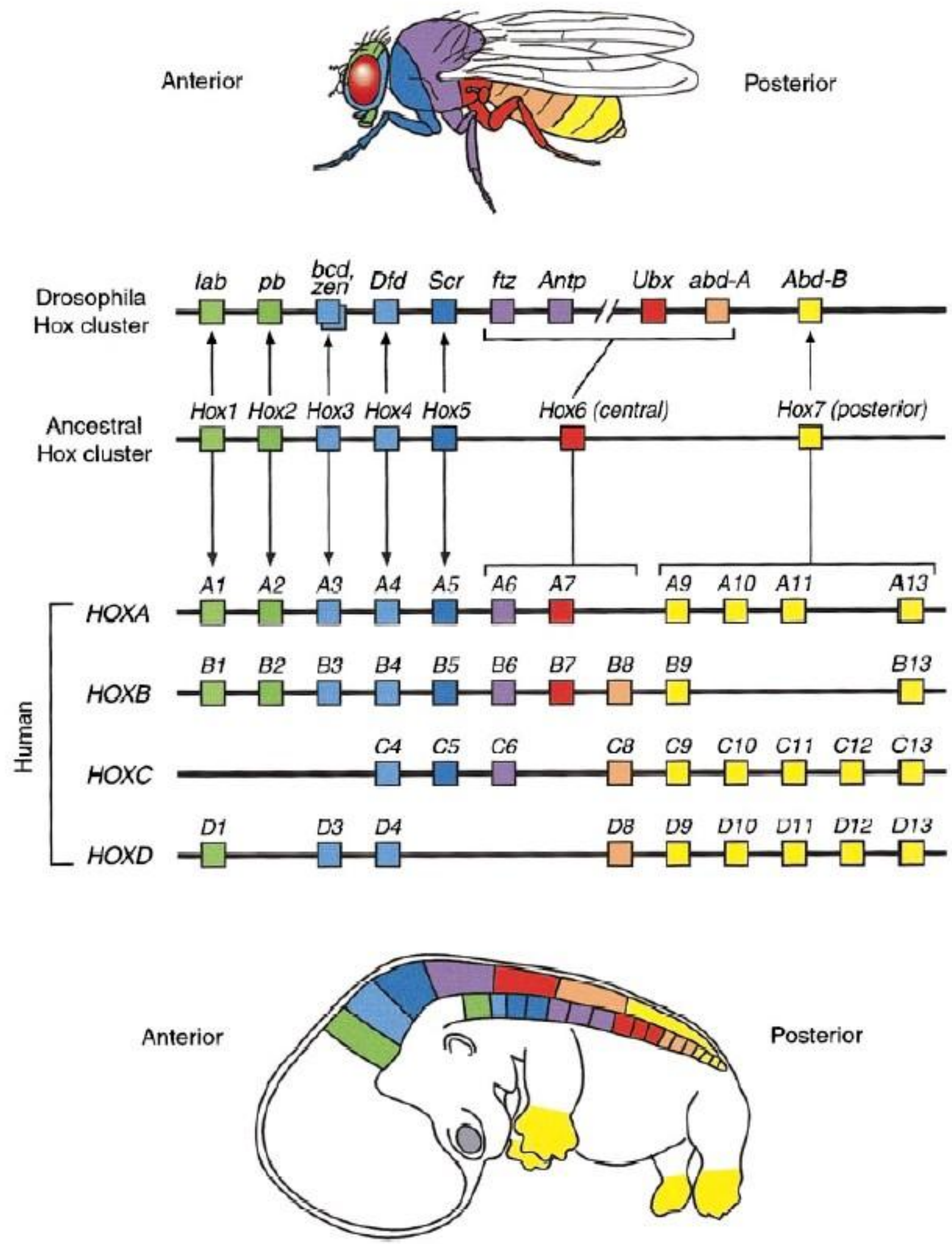
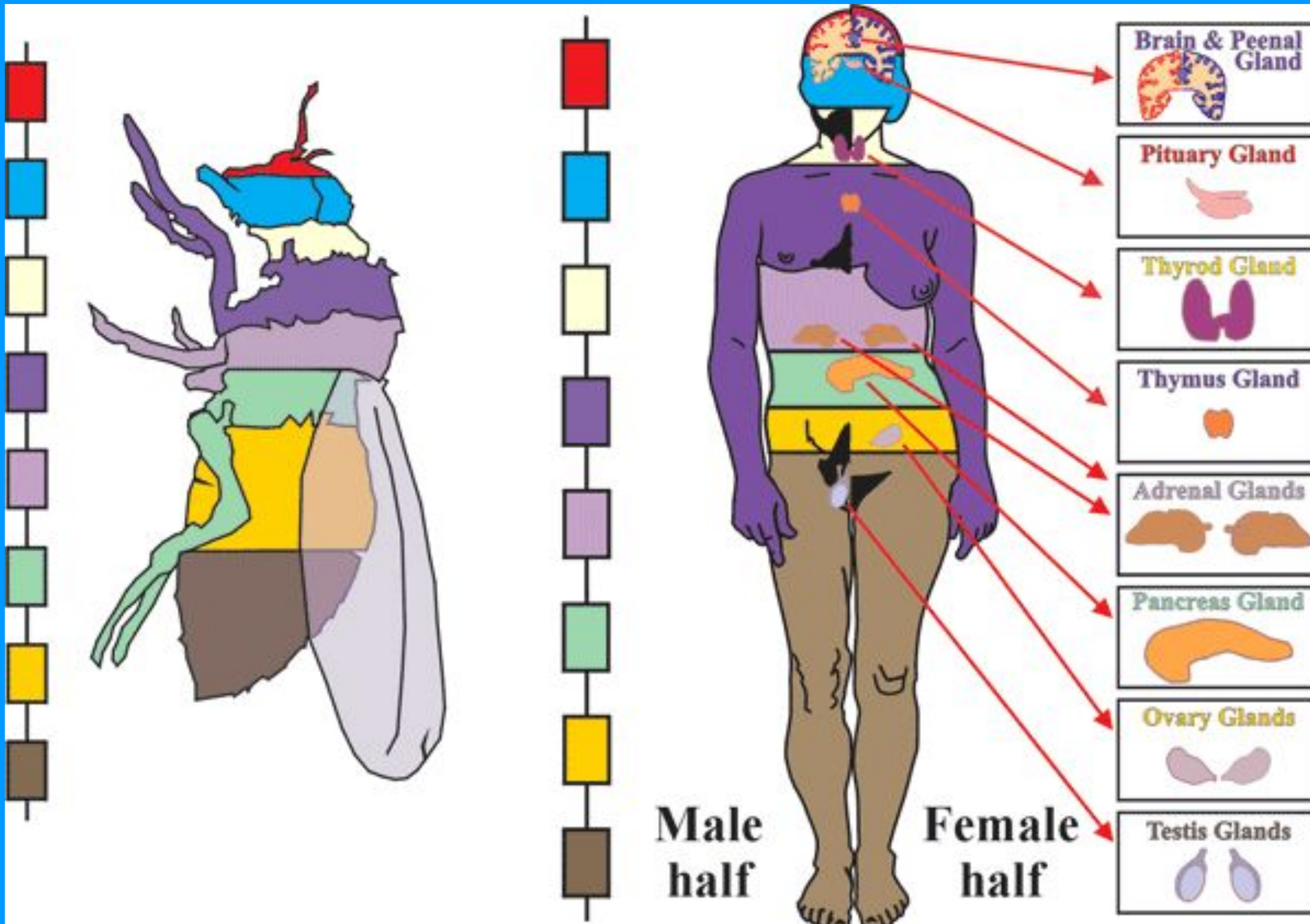


Figure 22.11
Postulated ancestry of the homeotic genes from a hypothetical ancestor of both deuterostomes and prototomes. Amphioxus has only one cluster, similar to that of insects. Vertebrates have four clusters, none of which is complete. (After Holland and Garcia-Fernández 1996.)


Anterior
Posterior
Drosophila
Hox cluster
lab
pb
bcd,
zen
Dfd
Scr
ftz
Antp
Ubx
abd-A
Abd-B
Ancestral
Hox cluster
Hox1
Hox2
Hox3
Hox4
Hox5
Hox6 (central)
Hox7 (posterior)
Human
HOXA
A1
A2
A3
A4
A5
A6
A7
A9
A10
A11
A13
HOXB
B1
B2
B3
B4
B5
B6
B7
B8
B9
B13
HOXC
C4
C5
C6
C8
C9
C10
C11
C12
C13
HOXD
D1
D3
D4
D8
D9
D10
D11
D12
D13
Anterior
Posterior



Brain & Peenal Gland
Pituary Gland
Thyrod Gland
Thymus Gland
Adrenal Glands
Pancreas Gland
Ovary Glands
Testis Glands
Male half
Female half

A summary of gene activity during *Drosophila* development

Hierarchy of Gene Activity in Early *Drosophila* Development

Maternal effect genes (egg-polarity genes)

↓

Gap genes

↓

Pair-rule genes

↓

Segment polarity genes

↓

Homeotic genes of the embryo

↓

Other genes of the embryo

Segmentation genes of the embryo

Homeobox & Homeodomain

- ДНК связывающий модуль
- факторов транскрипции
- генов дифференцировки

- 180 пн

- 60 аминокислотных остатков

Гомеотические гены кодируют транскрипционные факторы -ТФ, содержащие ДНК- связывающий гомеодомен (60 а.о.), имеющий структуру - спираль-поворот-спираль- Helix-Turn-Helix (HTH). Участок ДНК, кодирующий гомеодомен - гомеобокс (180 н.п.). Не все гомеобокс-содержащие гены - гомеотические, а только входящие в состав кластера генов - Hom-C. Все гомеодомен-содержащие ТФ одинаково узнают последовательность ТААТ, но различно фланкирующие ТААТ нуклеотиды.

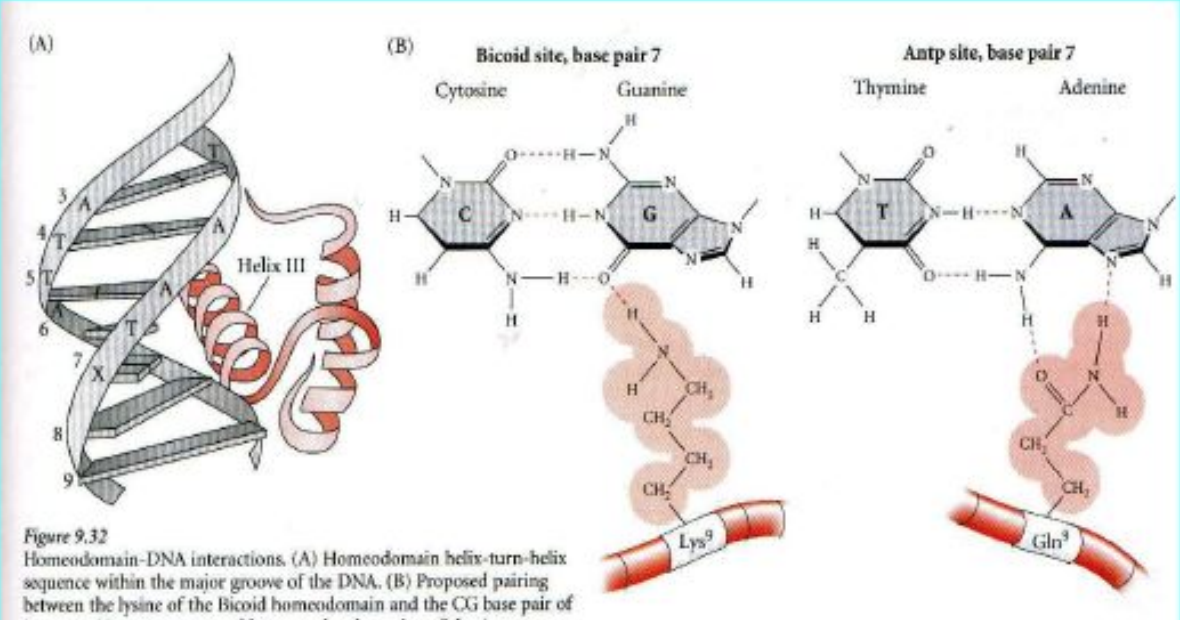
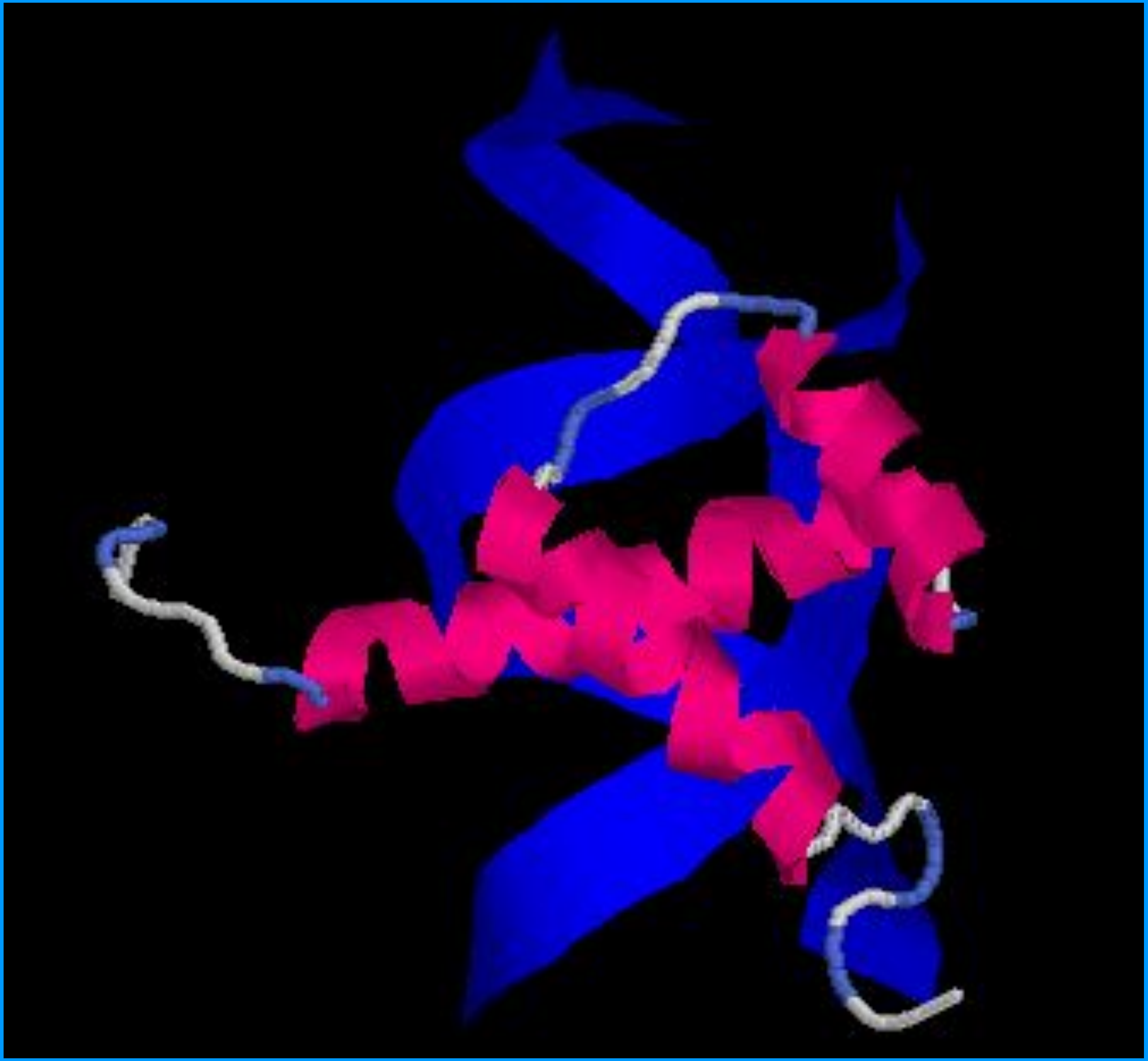
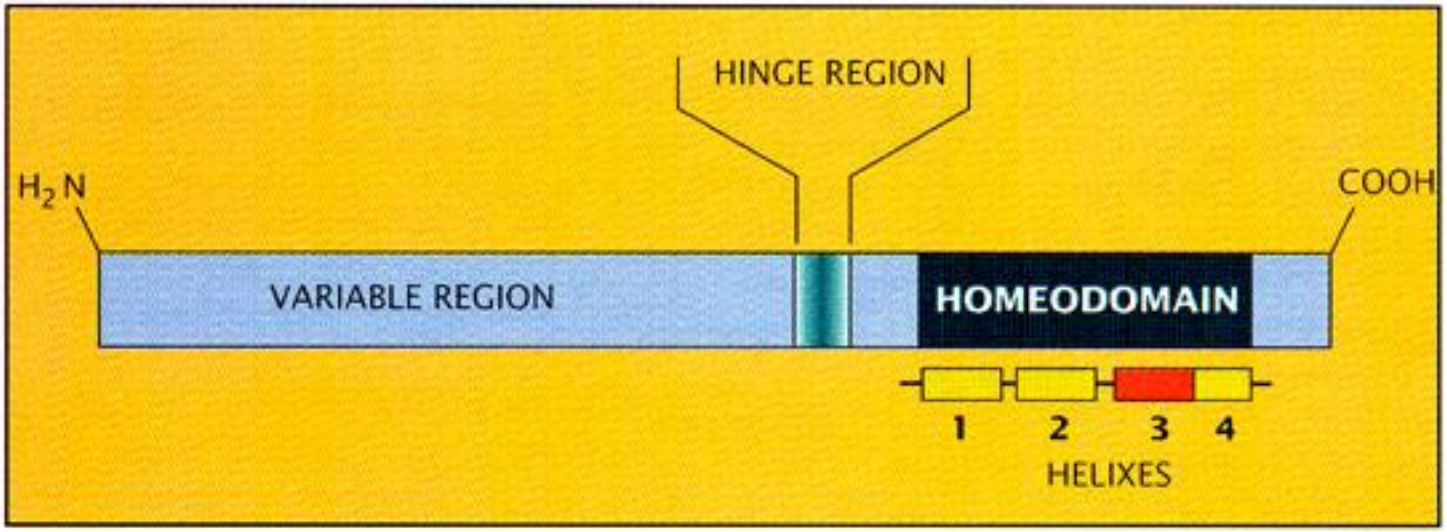


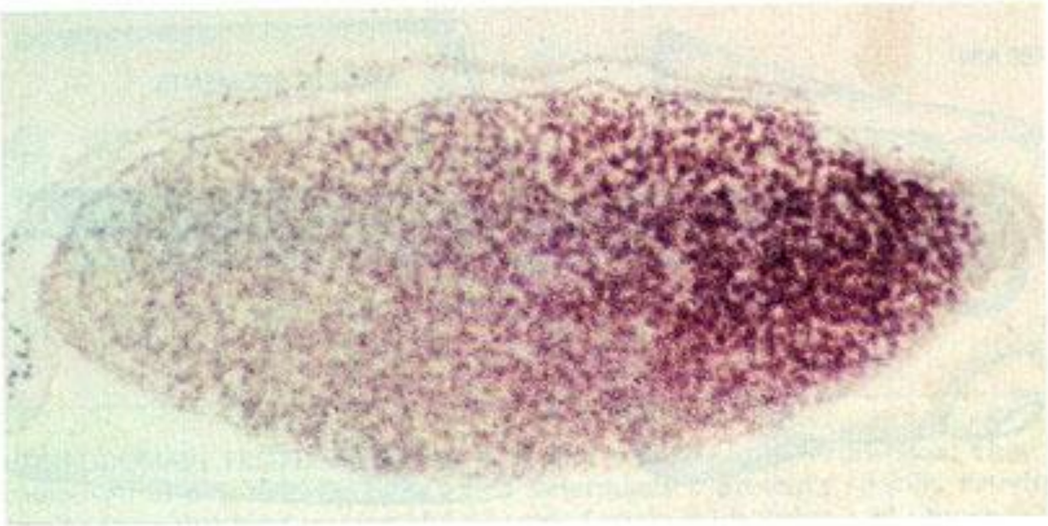
Figure 9.32
Homeodomain-DNA interactions. (A) Homeodomain helix-turn-helix sequence within the major groove of the DNA. (B) Proposed pairing between the lysine of the Bicoid homeodomain and the CG base pair of its recognition sequence, and between the glutamine of the Antennapedia homeodomain and the TA base pair of its recognition sequence. In both cases, the ninth amino acid of the homeodomain helix bonds with the base pair immediately following the TAAT sequence. (A after Riddihough 1992; B after Hanes and Brent 1991)

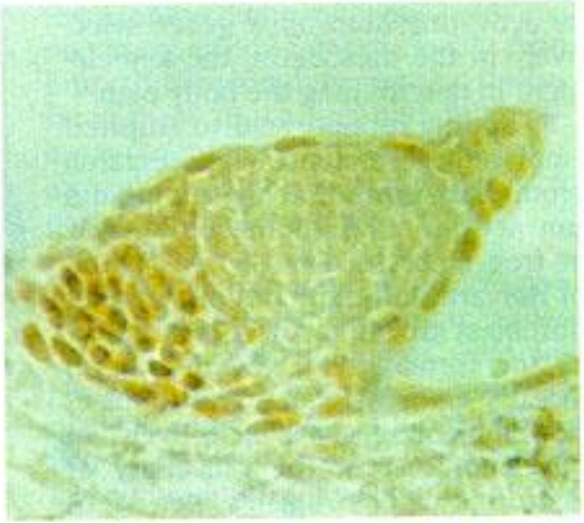


HOMEOBOX PROTEIN VND / DNA COMPLEX (1NK2)



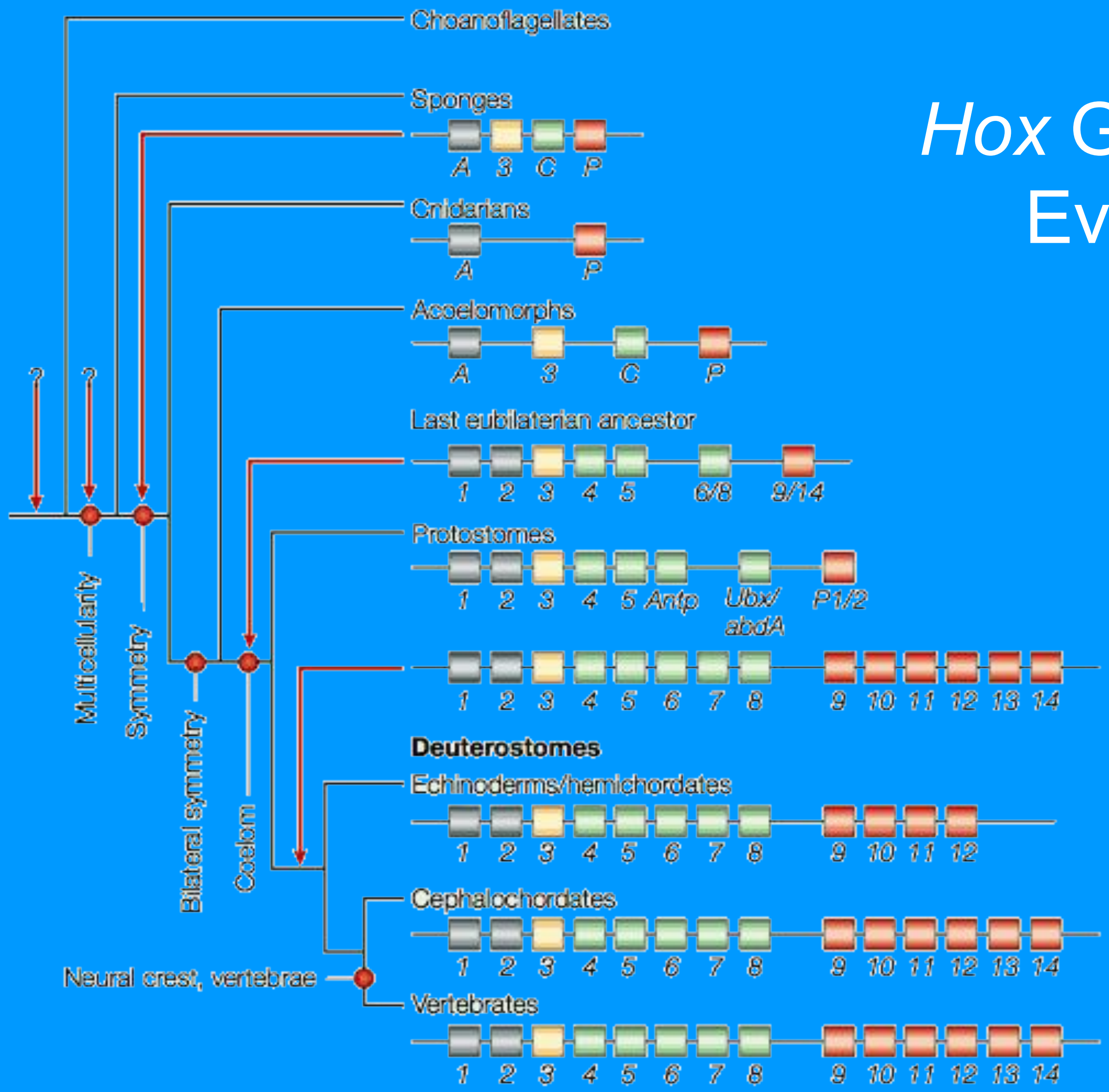
HOMEODOMAIN PROTEINS bind to DNA and regulate gene expression. They are composed of a variable region, which determines a protein's specific activity, a small connective hinge region and a homeodomain, a 60 amino acid sequence that is similar in all proteins of this type. This sequence is encoded by the homeobox regions of genes. The homeodomain consists of four alpha helixes (*1–4*), one of which (*red*) recognizes and binds to a specific DNA sequence in the target genes.



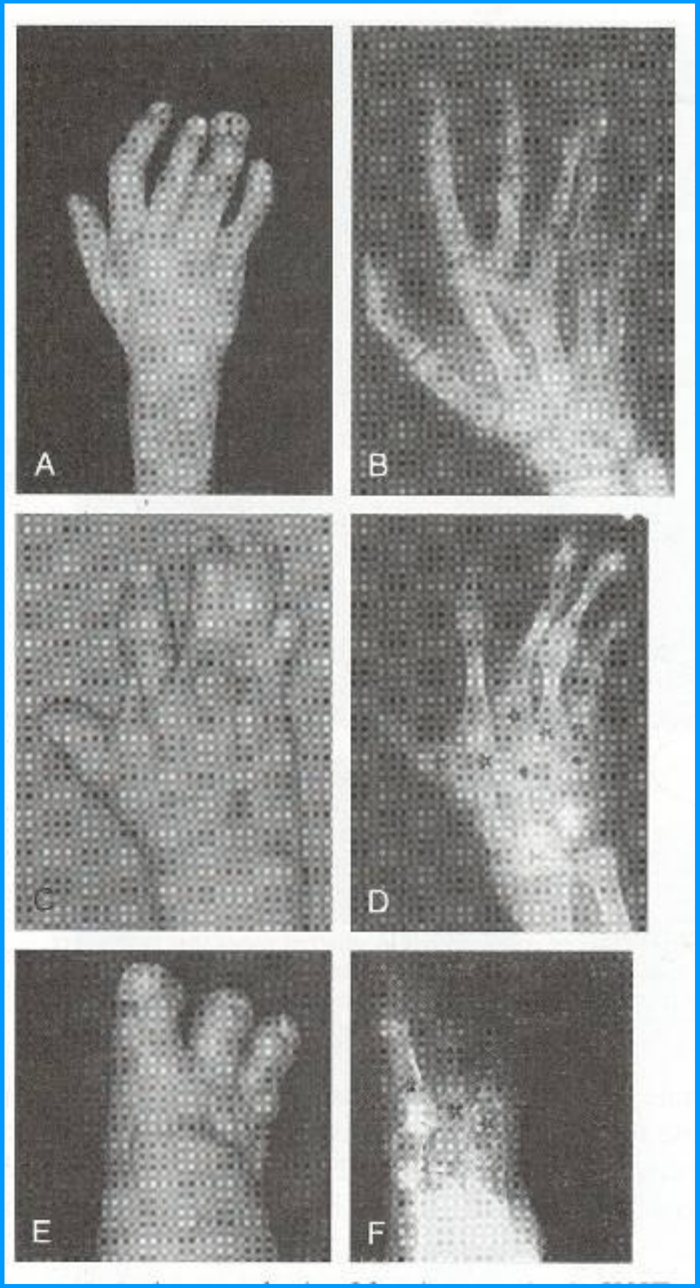


GRADIENTS of homeodomain proteins can be seen in these stained limb buds. In the chick wing bud (*left*), the concentration of Hox 5.2 protein is highest in the cell nuclei near the posterior region (*right edge*). In the pectoral fin bud of a zebrafish (*right*), the concentration of XlHbox 1 protein is highest in the anterior region (*left edge*). The fish pectoral fin is the evolutionary precursor of the tetrapod forelimb. Gradients are efficient mechanisms for conveying positional information.

Hox Genes and EvoDevo



A= anterior
Group 3
C= Central
P=Posterior



Mutation in HoxD13—synpolydactyly
Extra digits & interphalangeal webbing (hetero)
Similar but more severe & bony malformation of hands, wrists (Homo)

Loss of function-mild
Gain of function (Polyalanine expansion in HoxD13) similar to multiple loss of function-HoxD11,12,13

Polyalanine expansion-
Interfere all three proteins
Semidominant effect

Effect of very high doses of retinoic acid on Hox gene expression in mouse embryos

Normal mouse embryo

Retinoic acid: loss of many vertebrae

More retinoic acid: no posterior region formed

Vitamin A has caused the homeotic HOX genes 1-4 to become expressed in groups of cells that usually do not express these genes. It is well known that high doses of vitamin A taken by women early during pregnancy can disturb the regulation of HOX genes even in the human embryo and cause severe malformations.

Hierarchy of genes in *Drosophila* development

Maternal factor

↓

Gap genes

↓

Pair rule genes

↓

Selector genes (*Hox* genes)

↓

Realisator genes

Development of the number of segments

Development of the features of the segments

Hox genes in the Animal Kingdom