Developmental Genetics

May 25, 2016

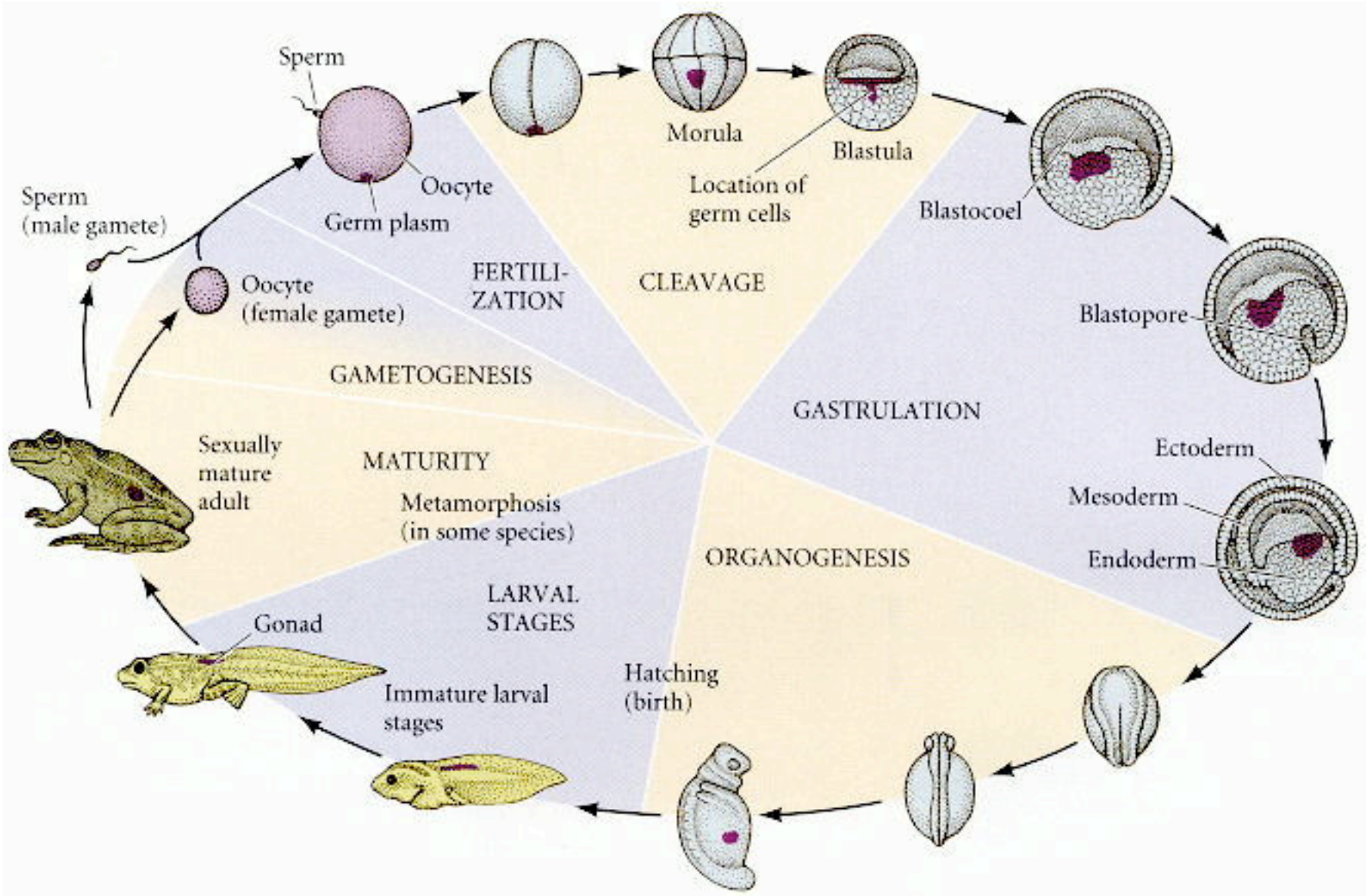
Developmental genetics

- development and cell differentiation
- positional information
- *Drosophila* model
- Hox genes and the conserved developmental toolkit

development in multicellular organisms:

- development is the process by which an adult organism is built from a fertilized egg
- every cell has the same genome, but ends up performing distinct functions
- the process of differentiation and specialization produces unique cell types, tissues, organs, organ systems, etc.
- this is accomplished largely through differential gene regulation, i.e. shutting off certain genes while activating others

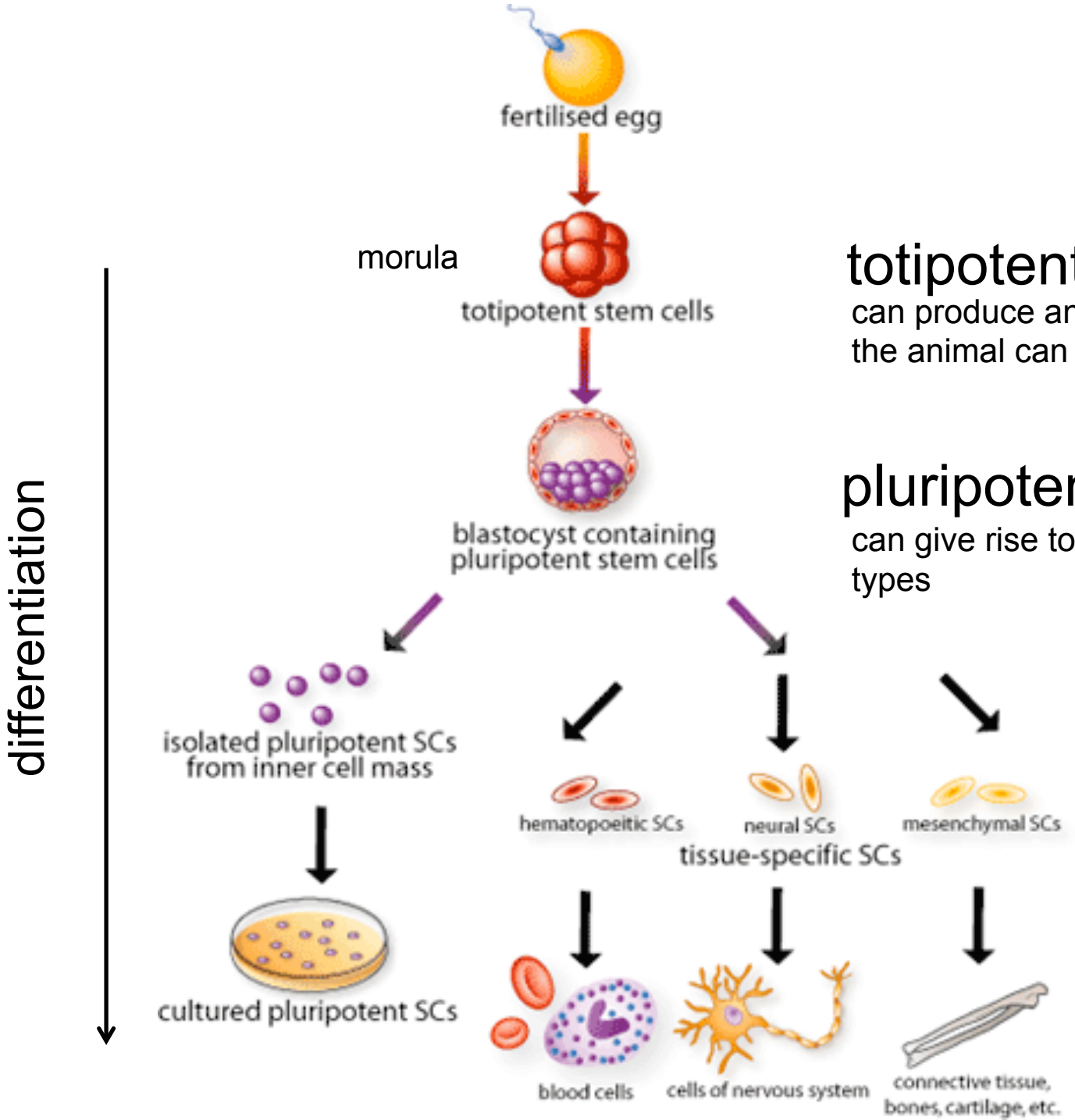
Development in *Xenopus laevis*



Gilbert, Developmental Biology, 4rd ed.

cell differentiation

- the process of **differentiation** involves changes in gene expression which progressively narrows down the types of genes expressed
- Most cells of adult animals are fully differentiated and locked into a specific cell fate
- However, there are some exceptions; pluripotent stem cells are found in a number of tissues
- These retain their ability to develop into a range of specialized cells to replenish those that are lost



totipotent

can produce any cell type or tissue the animal can produce

pluripotent

can give rise to most but not all cell types

multipotent

can give rise to a limited number of cell types

Adult stem cells are multipotent

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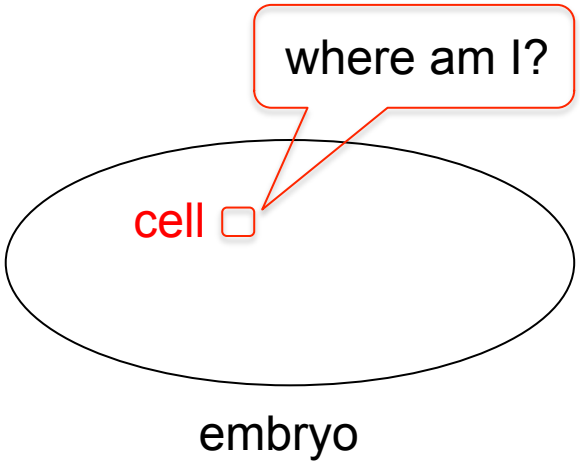
positional information

- **Positional information** is the term for the cues and signals that together inform a cell about its relative position in the embryo



positional information can be conveyed through **morphogen gradients**

- a **morphogen** is a substance whose presence in *different concentrations directs different developmental fates*

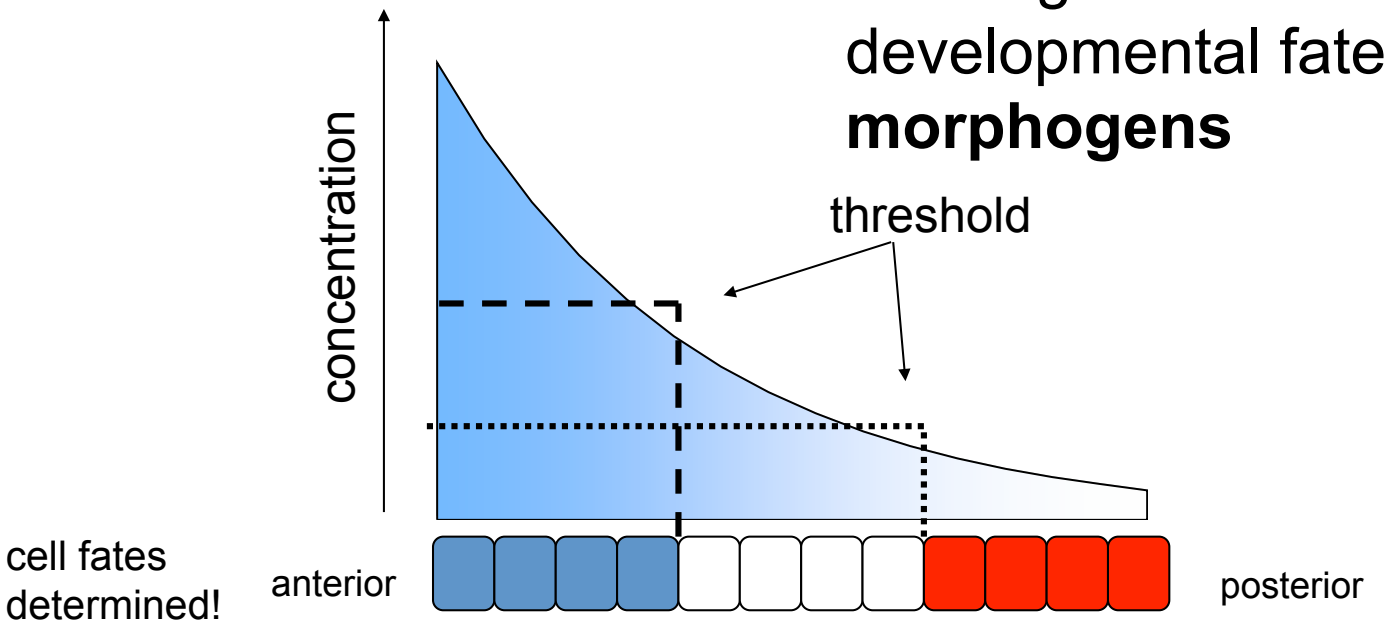
- idea of “formulative substances” such as this were originally proposed by Morgan (1901) and developed by Bovieri (1901)

- Turing further developed these ideas and coined the term “morphogen” (1952)

- Morphogen gradients were further developed and championed by Wolpert in the 1960’s, who proposed the “French Flag Model” of morphogenesis

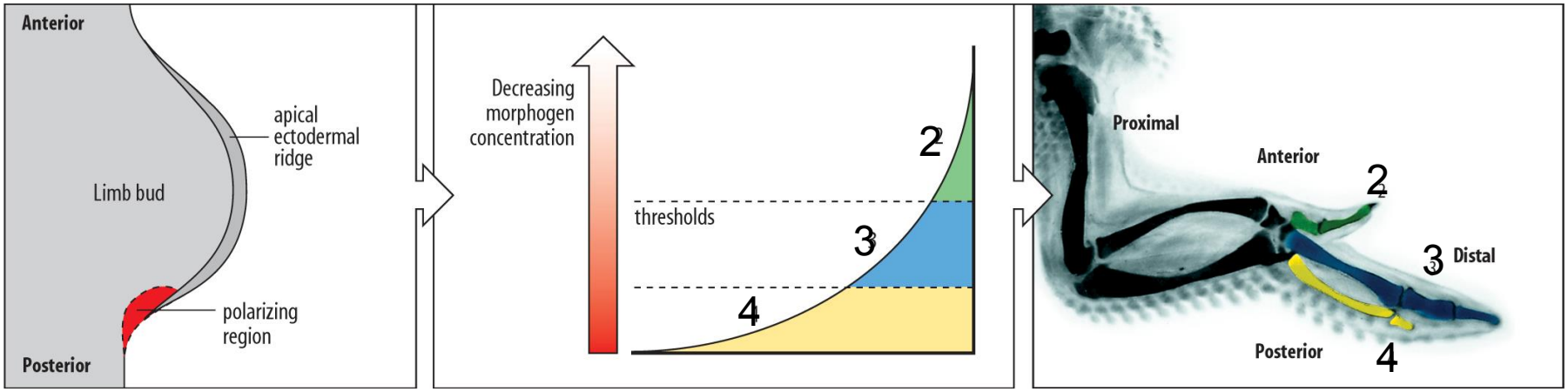
French flag model

- the **concentration of a molecule in space** can **determine cell fate**
- at high concentrations, cells will adopt a “blue” fate; at intermediate concentrations, cells will adopt a “white” fate; at low concentrations, cells will adopt a “red” fate
- Substances whose presence in differing concentrations directs developmental fates are called **morphogens**



first morphogen discovered in chick limb development

wild-type

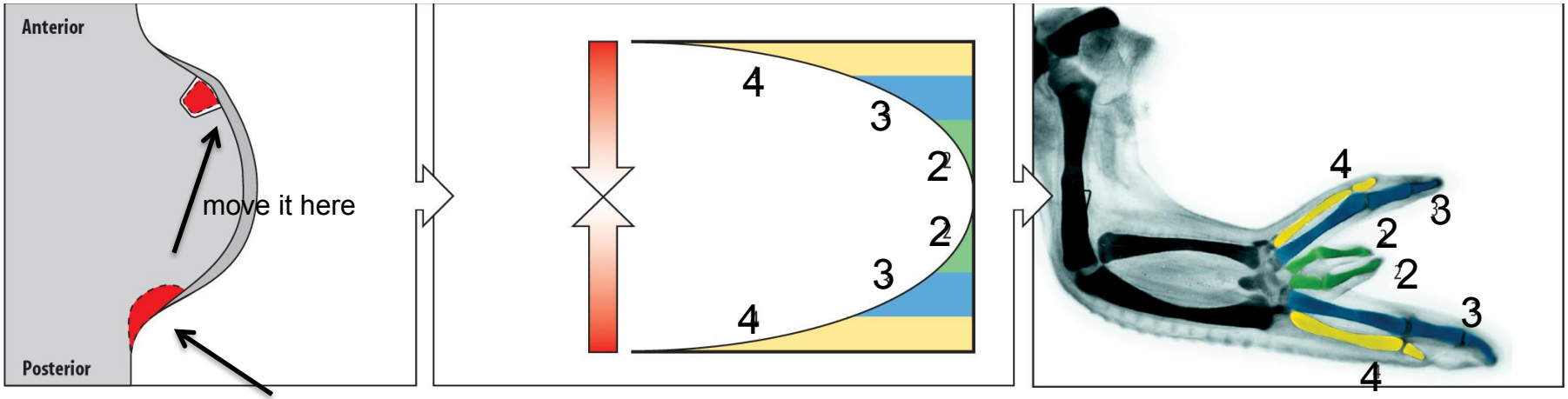


limb bud- embryonic structure that will develop into limb

morphogen concentration

wing phenotype

mutant



take a bit from this region, producing the morphogen

experiment from MacCabe, Gassling, and Saunders (1973)

NATURE VOL. 327 18 JUNE 1987

Embryology

We have a morphogen!

J.M.W. Slack

THE first morphogen has been identified and it is retinoic acid. Of course nothing in science is really certain, so perhaps I should say that the article by Thaller and Eichele on page 625 of this issue[1] completes a strong *prima facie* case for the identity of the first morphogen. So what is a morphogen and why is this work so important?

development the pattern of digits, and indeed all the other structures in the limb, arise as a result of threshold responses by the cells at the appropriate morphogen concentrations.

retinoic acid implicated as the morphogen in chick limb development

Identification and spatial distribution of retinoids in the developing chick limb bud

Christina Thaller & Gregor Eichele

Department of Physiology and Biophysics, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115, USA

DEVELOPMENTAL BIOLOGY

We may not have a morphogen

Jeremy Brockes

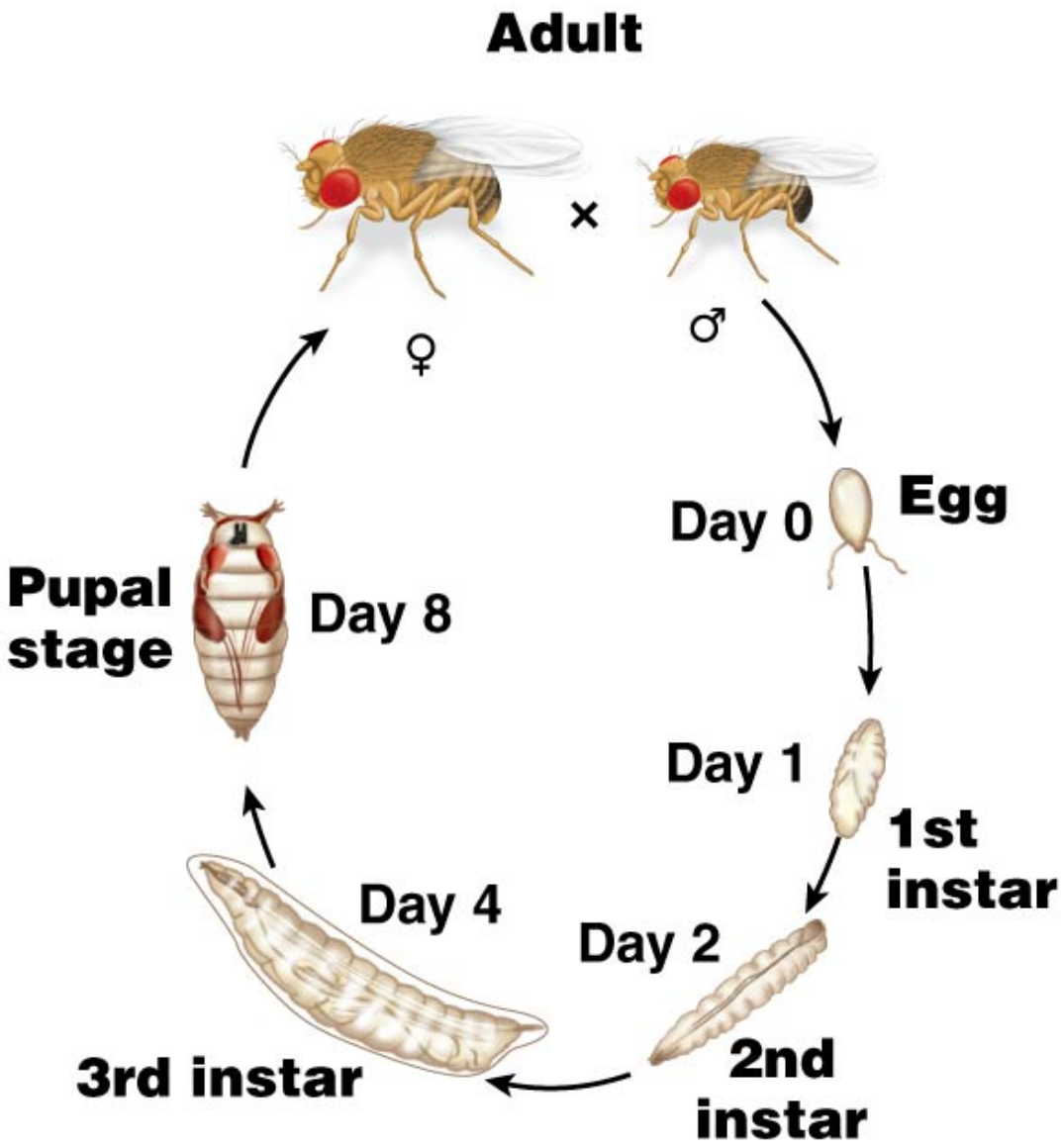
ATTENTION has centred on retinoic acid (RA) as a potential vertebrate morphogen because of its ability to alter axial specification in avian limb development and urodele limb regeneration (reviewed in refs 1–3). In signalled to produce more RA, thereby retaining its status as a possible morphogen.

Noji *et al.*[5] provide evidence against this second hypothesis, thus complementing the study of Wanek *et al.* These authors and

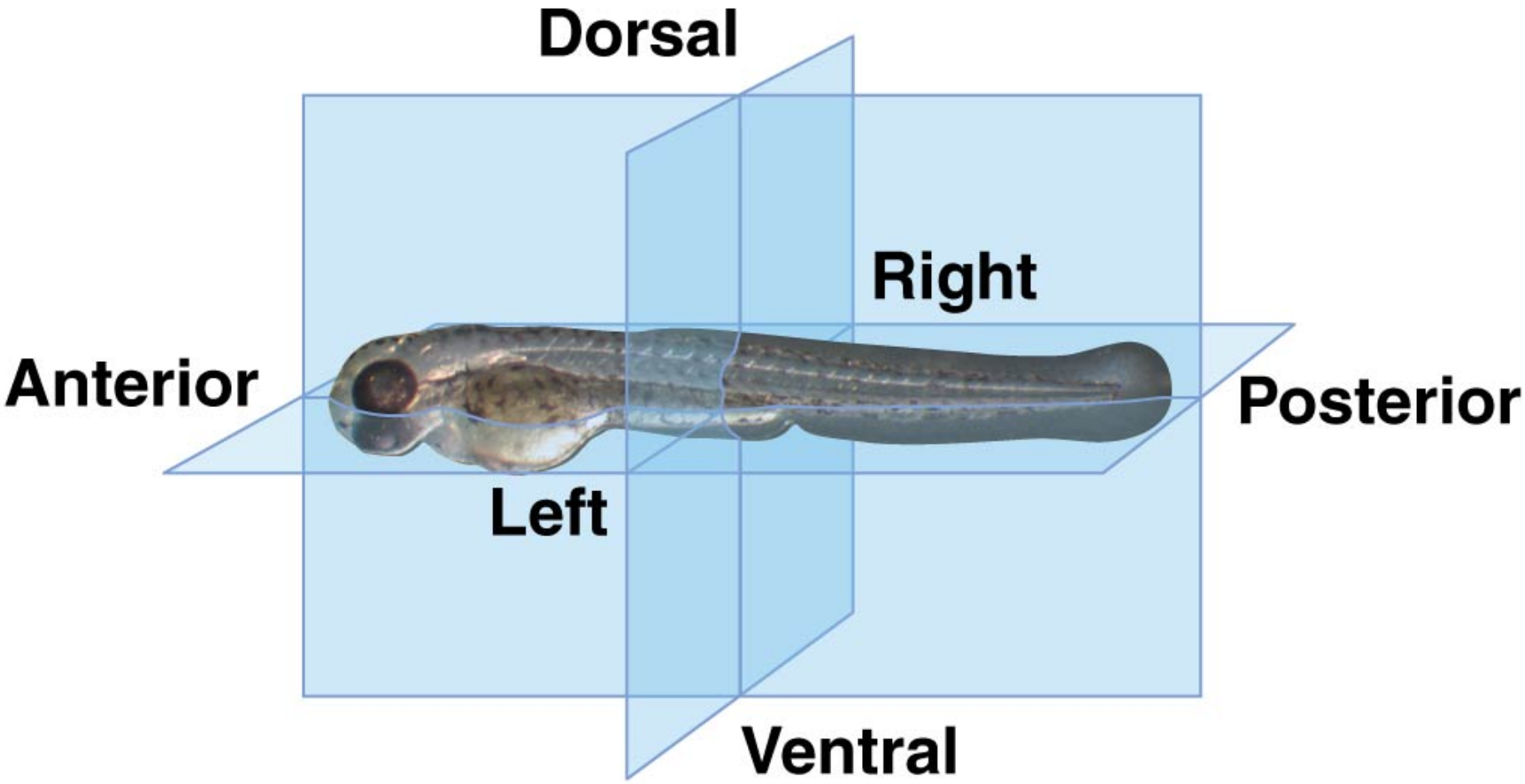
Developmental genetics

- development and cell differentiation
- pattern formation
- *Drosophila* model
  - life cycle
  - embryonic development
  - maternal effect genes
  - anterior-posterior axis formation (maternal coordinate, gap, pair-rule, segment polarity, and hox genes)
- Hox genes and the conserved developmental toolkit

(a) *Drosophila* life cycle



- *Drosophila* are a model species for animal development
- Development has been extensively studied in this model
- They have short generation times, going from egg to adult in ~10 days
- Egg to larva development occurs in only 24 hours

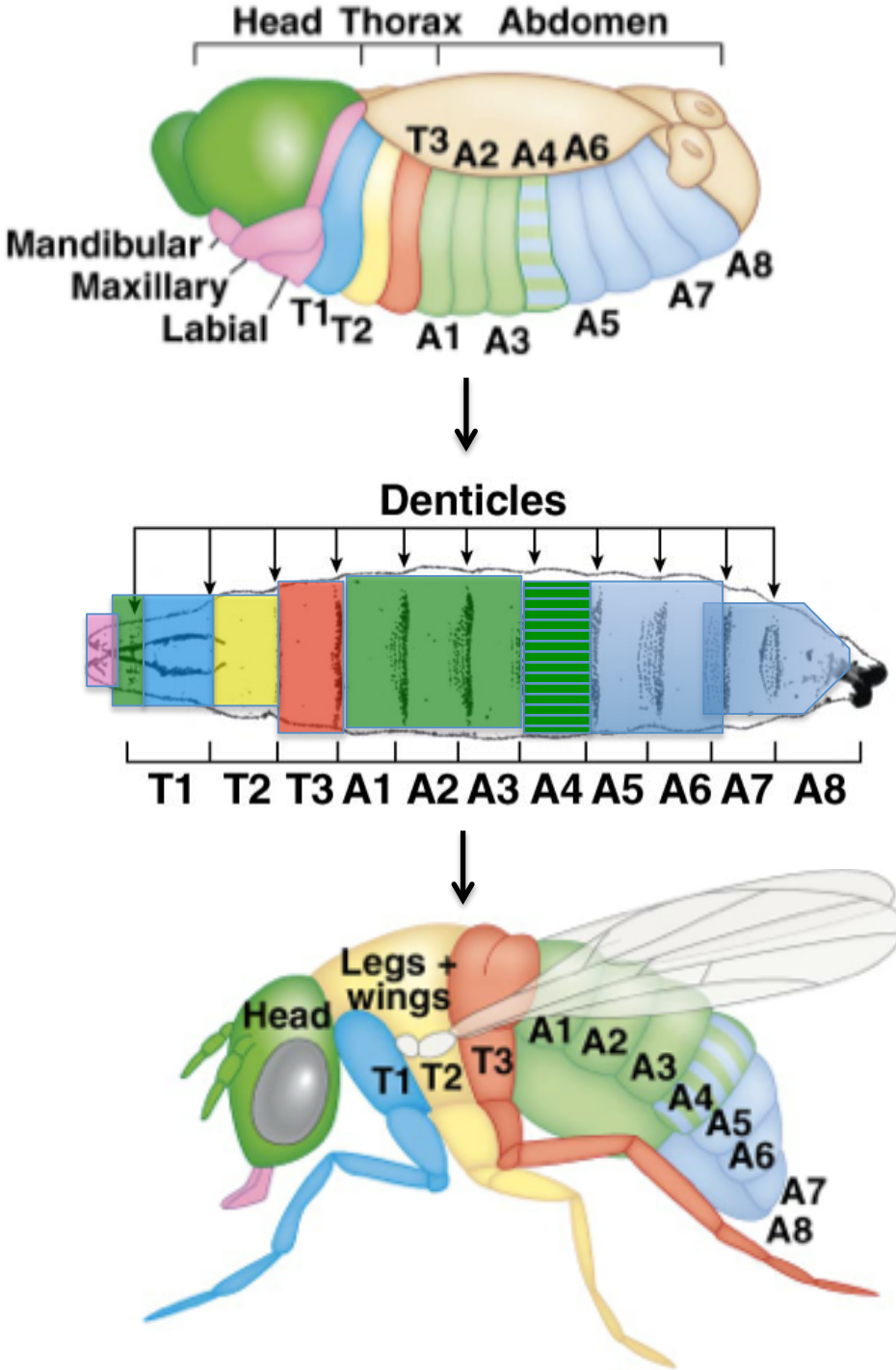


Body axes

anterior-posterior : head to tail

dorsal-ventral : back to front

left-right



Segmentation along the anterior-posterior axis

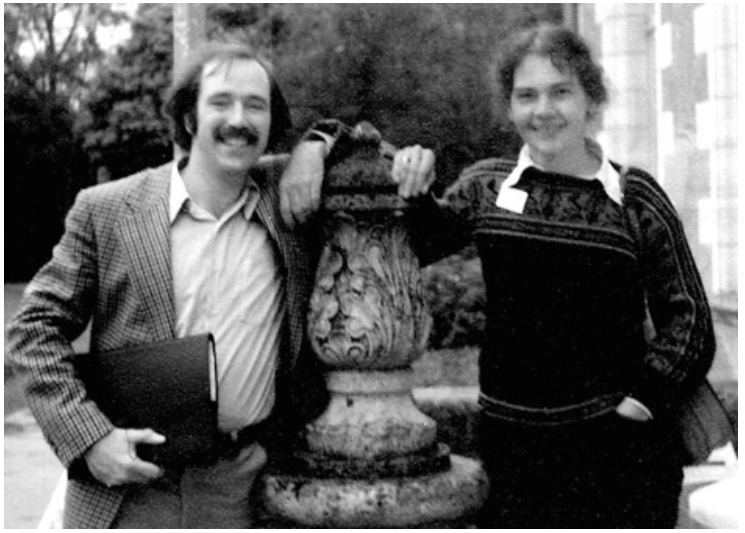
- Segments are determined during embryogenesis
- always the same number and position of segments
- these repeated segments carry through to larval stage
- during pupal stage, these segments are given distinct identities seen in adult

Mutations affecting segment number and polarity in *Drosophila*

Christiane Nüsslein-Volhard & Eric Wieschaus

European Molecular Biology Laboratory, PO Box 10.2209, 69 Heidelberg, FRG

Nature Vol. 287 30 October 1980



Wieschaus Nüsslein-Volhard

- conducted a mutant screen to identify genes involved in segmentation
- used a mutagen to induce mutations randomly throughout the genome, bred to create lines with only one mutation each

↓

Results of mutagenesis screens

Total lines established and tested	26978
Lethal mutations	18136
Mutations causing embryonic lethality	4332
Mutations causing embryonic phenotypes	580
Complementation Groups (Genes)	139

- looked for recessive embryonic lethals (embryos died when mutations were homozygous)
- characterized the mutant phenotypes of these genes
- discovered that these **mutant phenotypes** fell into **distinct categories**

Classes of mutations identified genes involved in segmentation

1. **Maternal genes**: mutants are missing an entire pole (head or tail)
2. **Gap genes**: mutants are missing large contiguous stretch of segments
3. **Pair-rule genes**: mutants are missing parts of alternating segments (every other segment)
4. **Segment-polarity genes**: defects affect patterning within each of the 14 segments
5. **Homeotic genes**: defects alter the identity of one or more segments

about these classifications…

- this classification of genes identifies **where they are expressed** in **time** (during development) and **space** (where physically in the embryo)
- the 5 classes of genes are expressed **in sequence** during embryogenesis
- most genes identified are **transcription factors**
- earlier expressed genes affect transcription of later expressed genes

gene category	expression pattern (of one gene in category)	function
Maternal		establish gradients from anterior to posterior poles of eggs
Gap		define broad regions in the egg
Pair-rule		define 7 segments
Segment polarity		define 14 segments
Homeotic		define identity of each segment, what adult structures will form

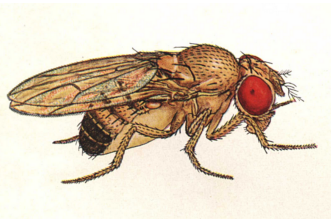
Maternal effects on pattern formation

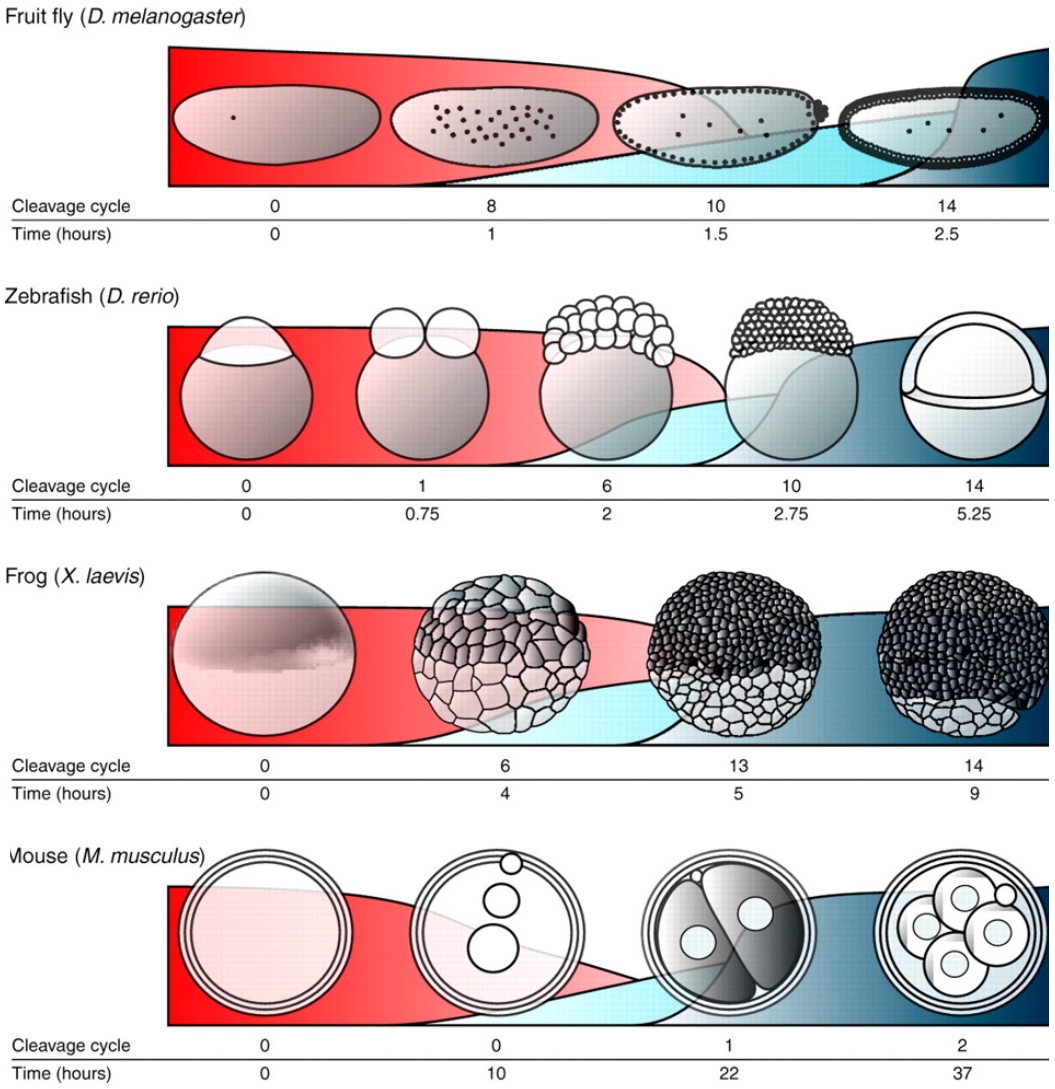
- **Maternal effect genes** encode products that the mother supplies to the egg to direct embryonic development

- These are supplied either as mRNA or as protein

- **Zygotic genes** are also involved in developmental processes; these are transcribed in the embryo (zygote)

The maternal to zygotic transition





maternal mRNA/ protein

early embryonic (zygotic) gene expression (just a few genes turn on)

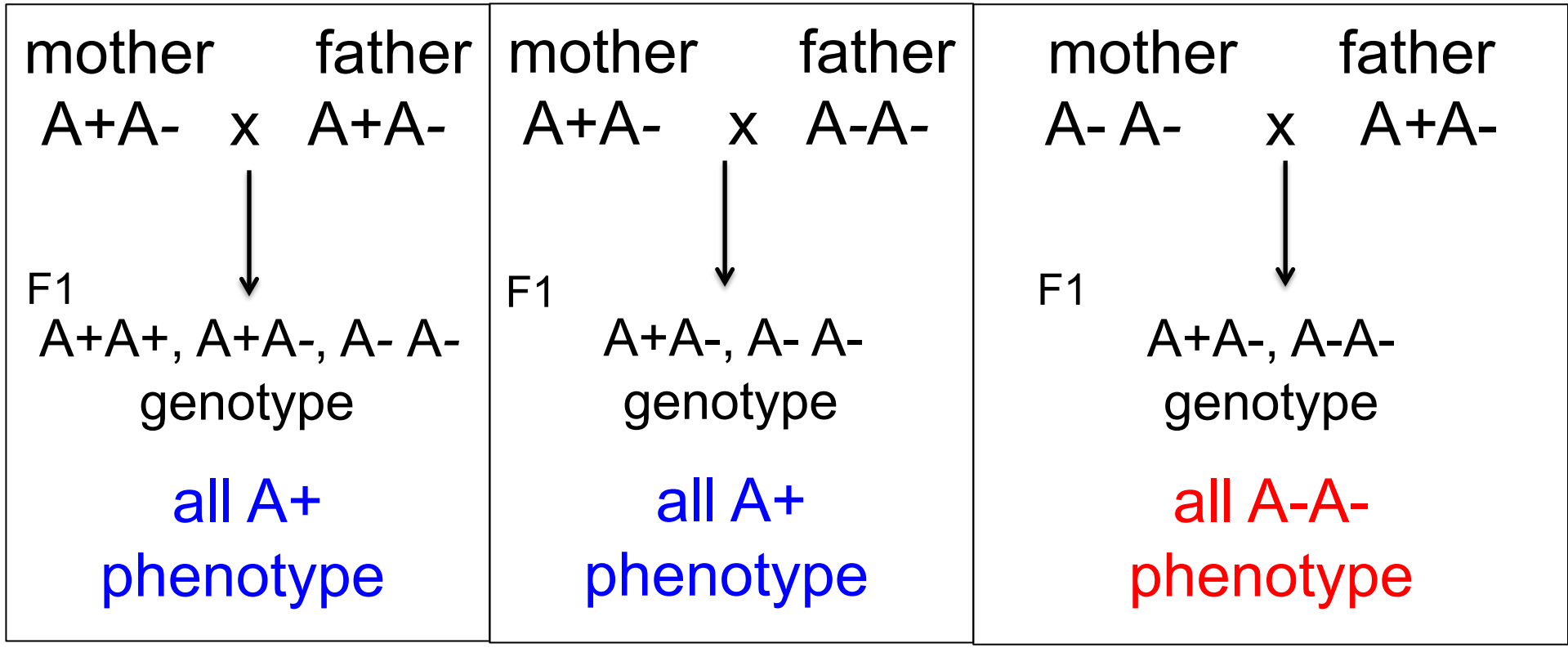
full zygotic expression activation (most of the zygotic genes turn on)

Tadros & Lipshitz, 2009

pattern of inheritance for maternal effect genes

- For maternal effect genes *the genotype of the mother determines the phenotype of the zygote*

example, autosomal recessive maternal effect gene
A+ is wild type, A- is mutant



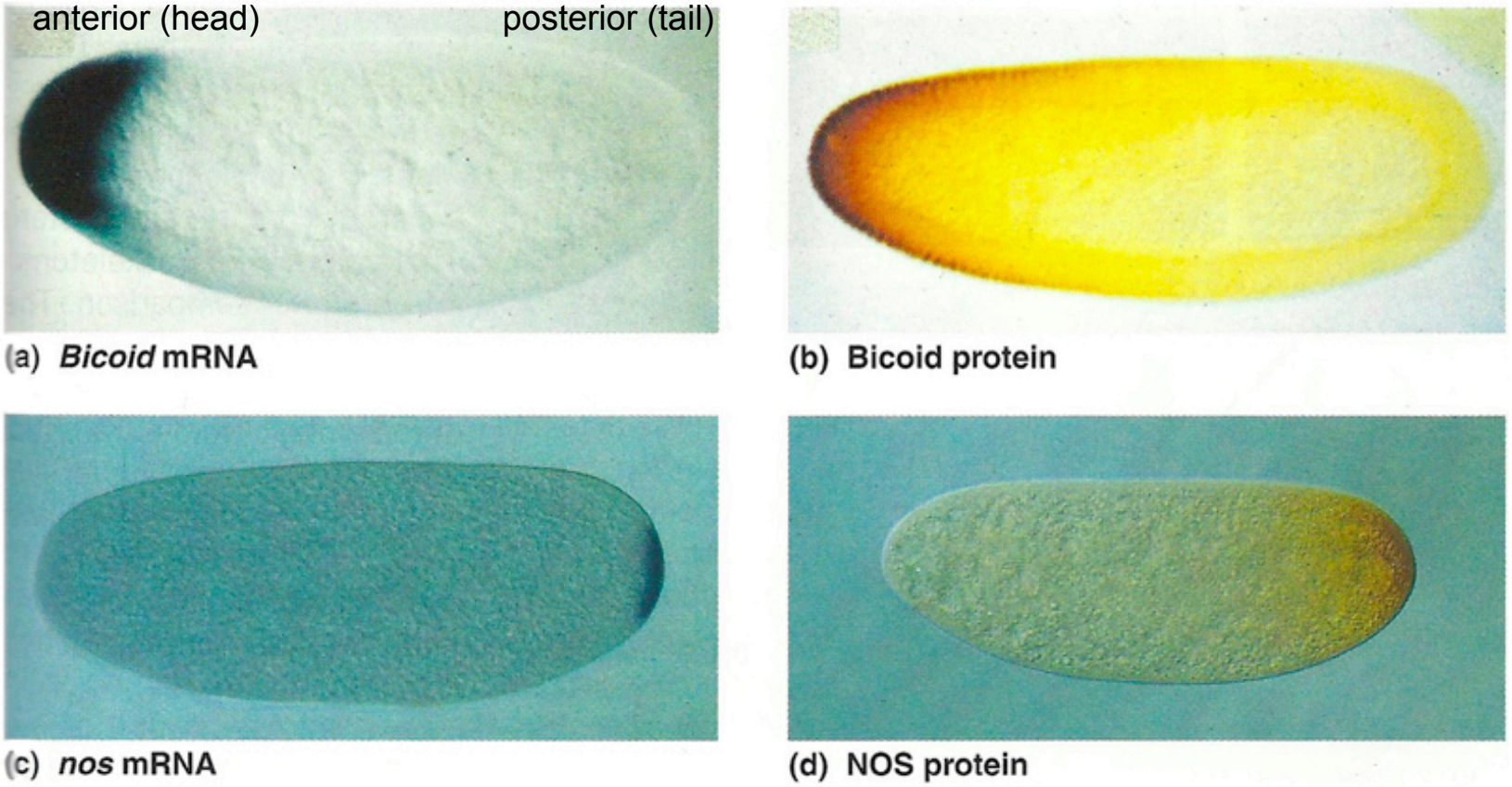
Maternally supplied gene products begin patterning of the anterior-posterior axis

- Most of the early acting genes that establish the anterior-posterior axis encode **transcription factors**

- Interaction of the transcription factors with regulatory sequences of target genes provides **spatial control of gene expression**

- Spatial and temporal control of gene expression results in subdivision of a *Drosophila* embryo into its characteristic segments

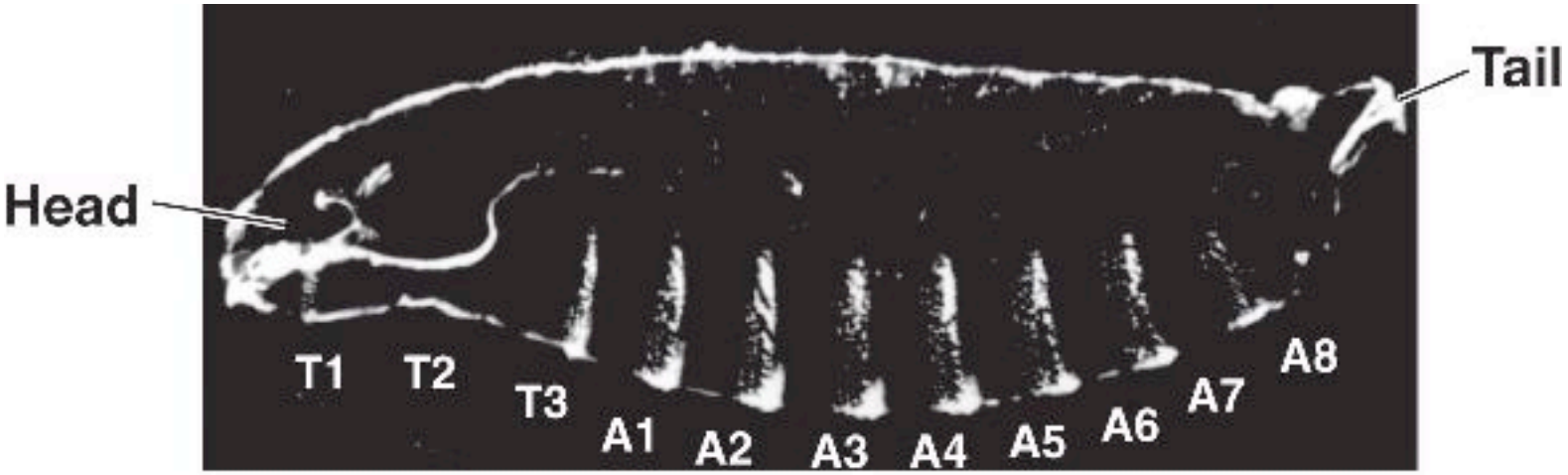
localized maternal mRNAs are translated into protein gradients



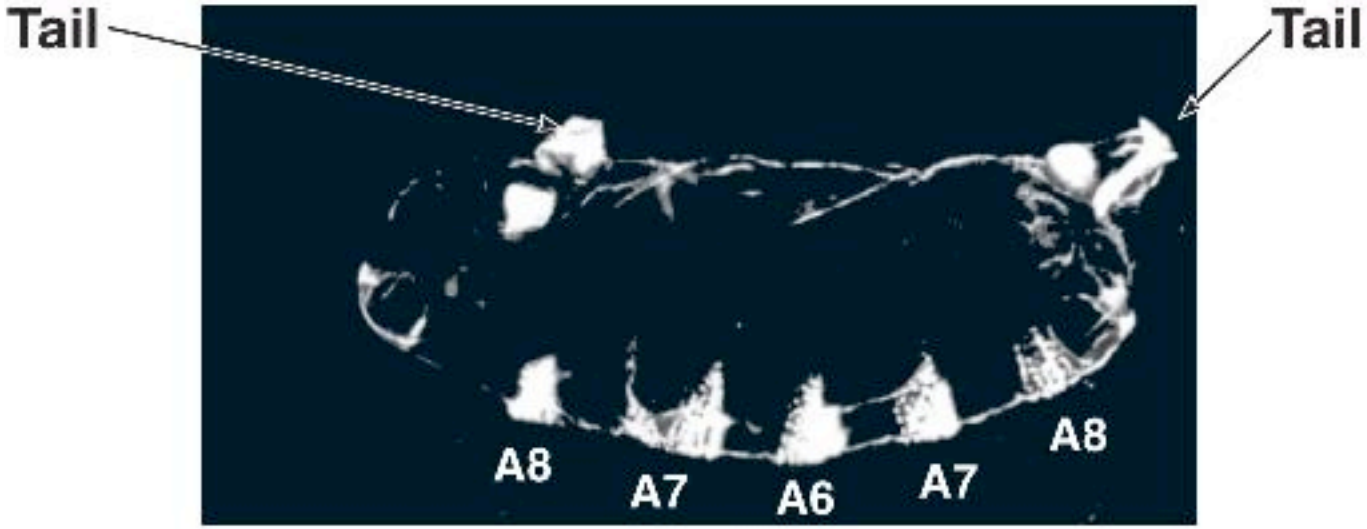
maternally deposited *bicoid* mRNA is localized at the anterior pole, diffuses into a gradient as it is translated into Bicoid protein

maternally deposited *nanos* mRNA is localized to posterior pole, diffuses into a gradient as it is translated into Nanos protein

maternal A-P axis gene mutants affect an entire pole of the larva



Wild-type

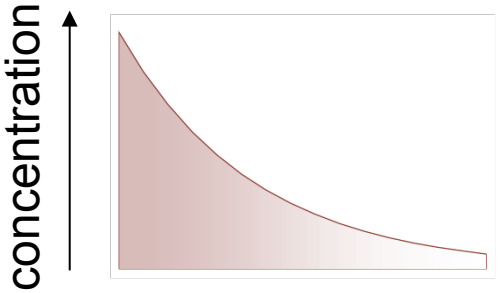


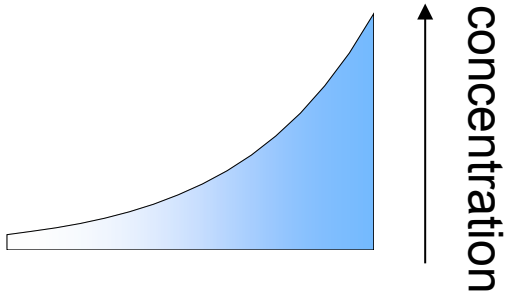
bicoid mutant

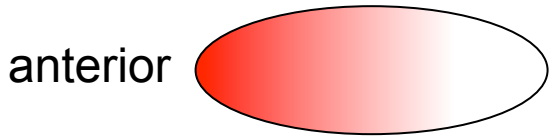
mothers homozygous for *bicoid* mutations produce embryos with <u>two tails</u> and <u>no head</u>

Are these factors morphogens, like in the French Flag model?

But do they affect cell fates in a concentration dependent manner?

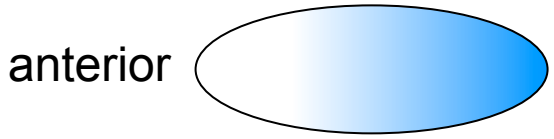






Bicoid

Yes, they do form concentration gradients…

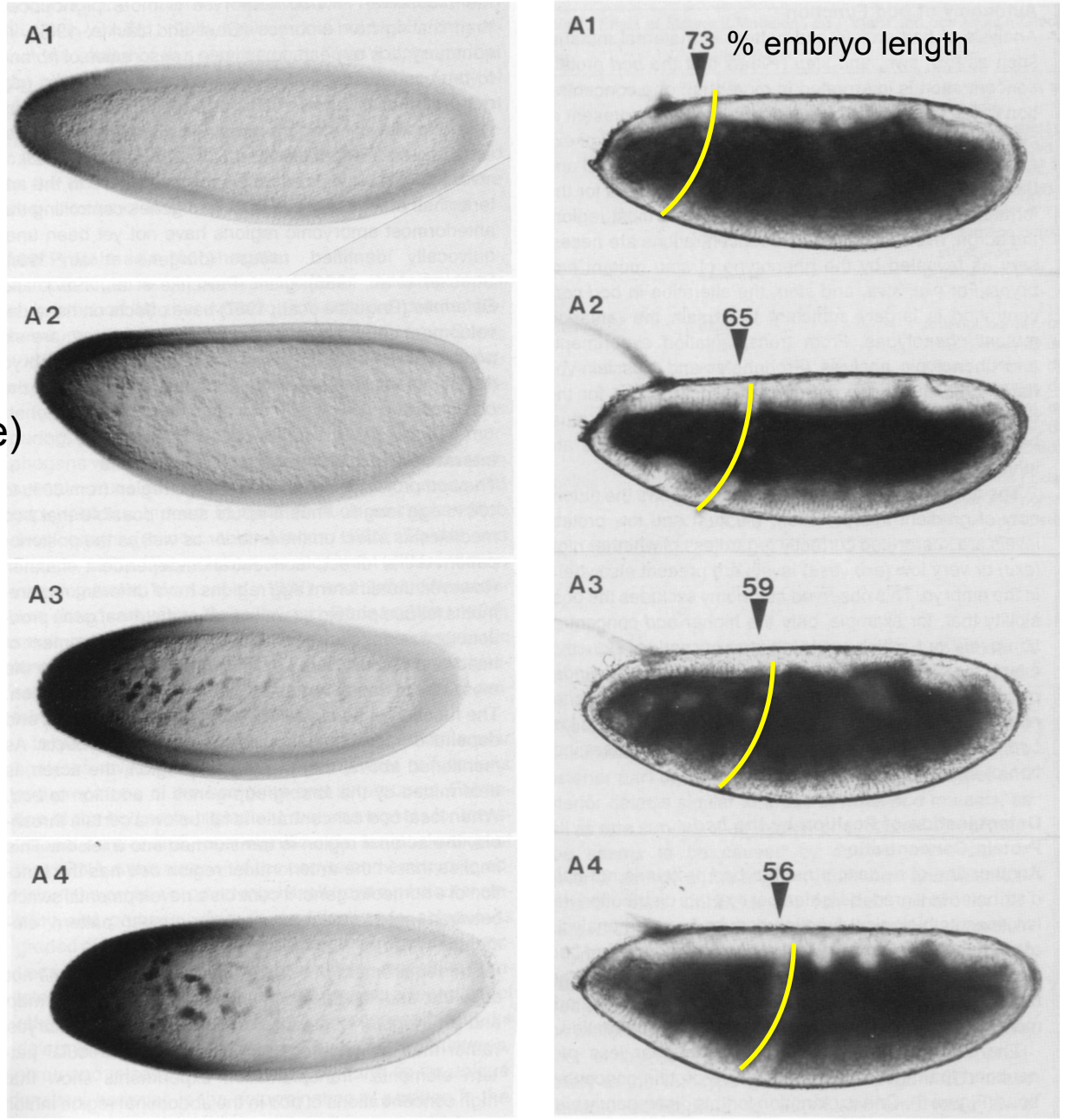


Nanos

copies of *bicoid* gene in mother

Bicoid protein

position of head fold



1

2 (wild-type)

3

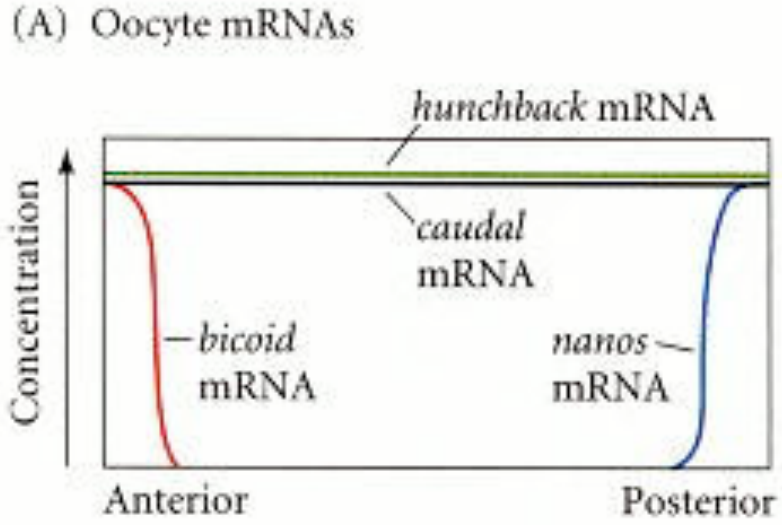
4

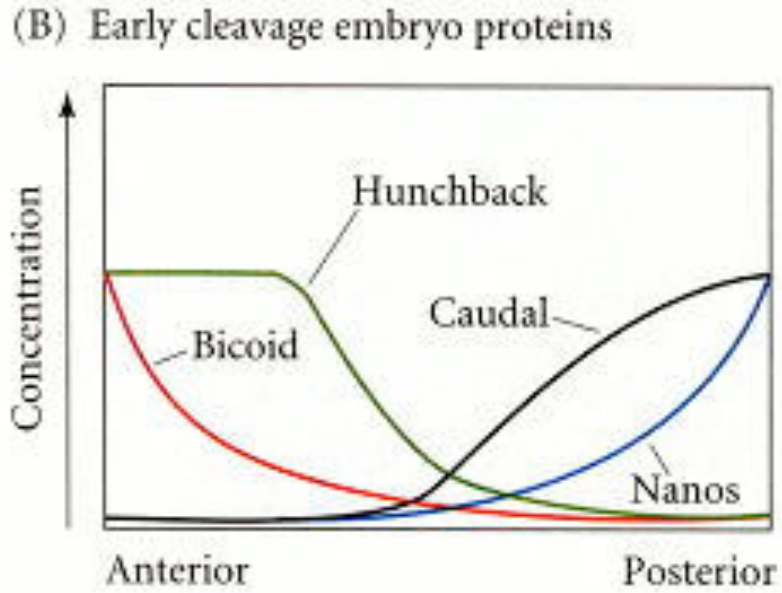
Bicoid determines anterior structures in a concentration-dependent manner

is a morphogen!

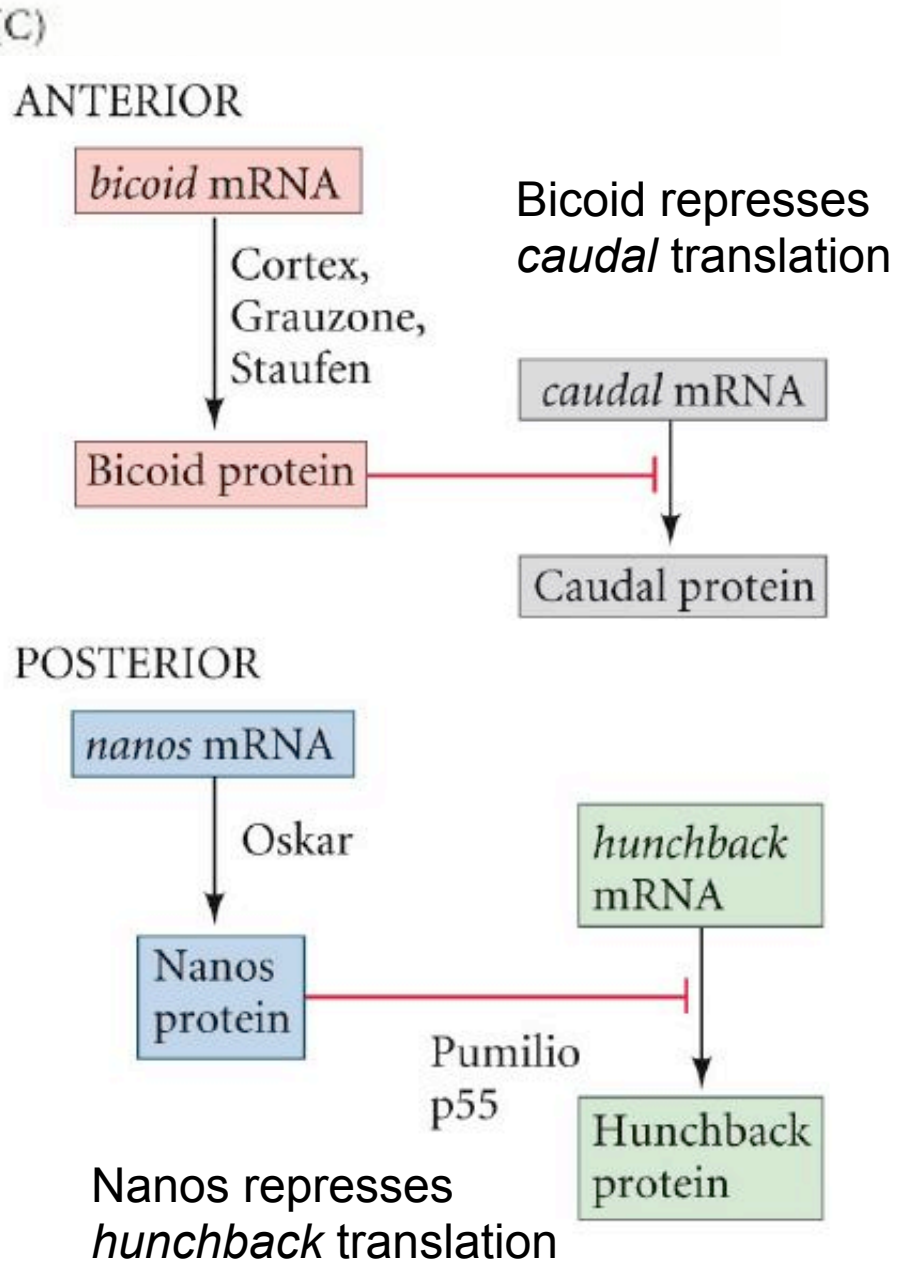
higher copy number = more *bicoid* = larger head region

Driever & Nusslein-Volhard (1988) *Cell*





embryo



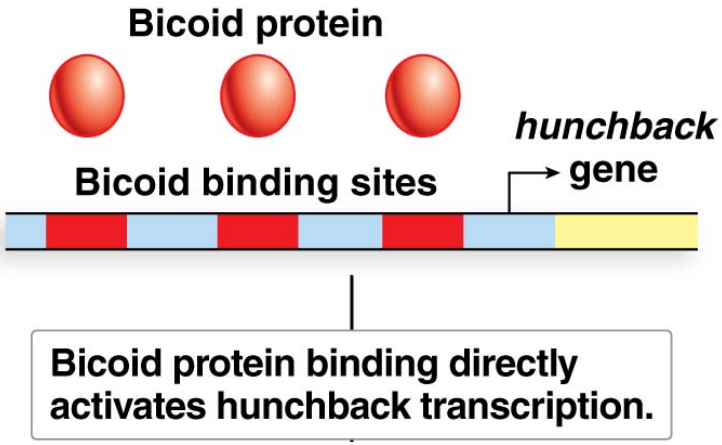
Bicoid represses *caudal* translation

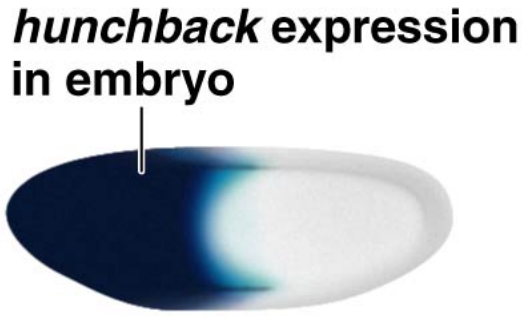
Nanos represses *hunchback* translation

Gilbert, Developmental Biology, 6th ed.

- these interactions between maternal factors are regulating translation
- the rest of the interactions in this pathway are regulating transcription
- for example, maternal genes activate or repress expression of gap genes

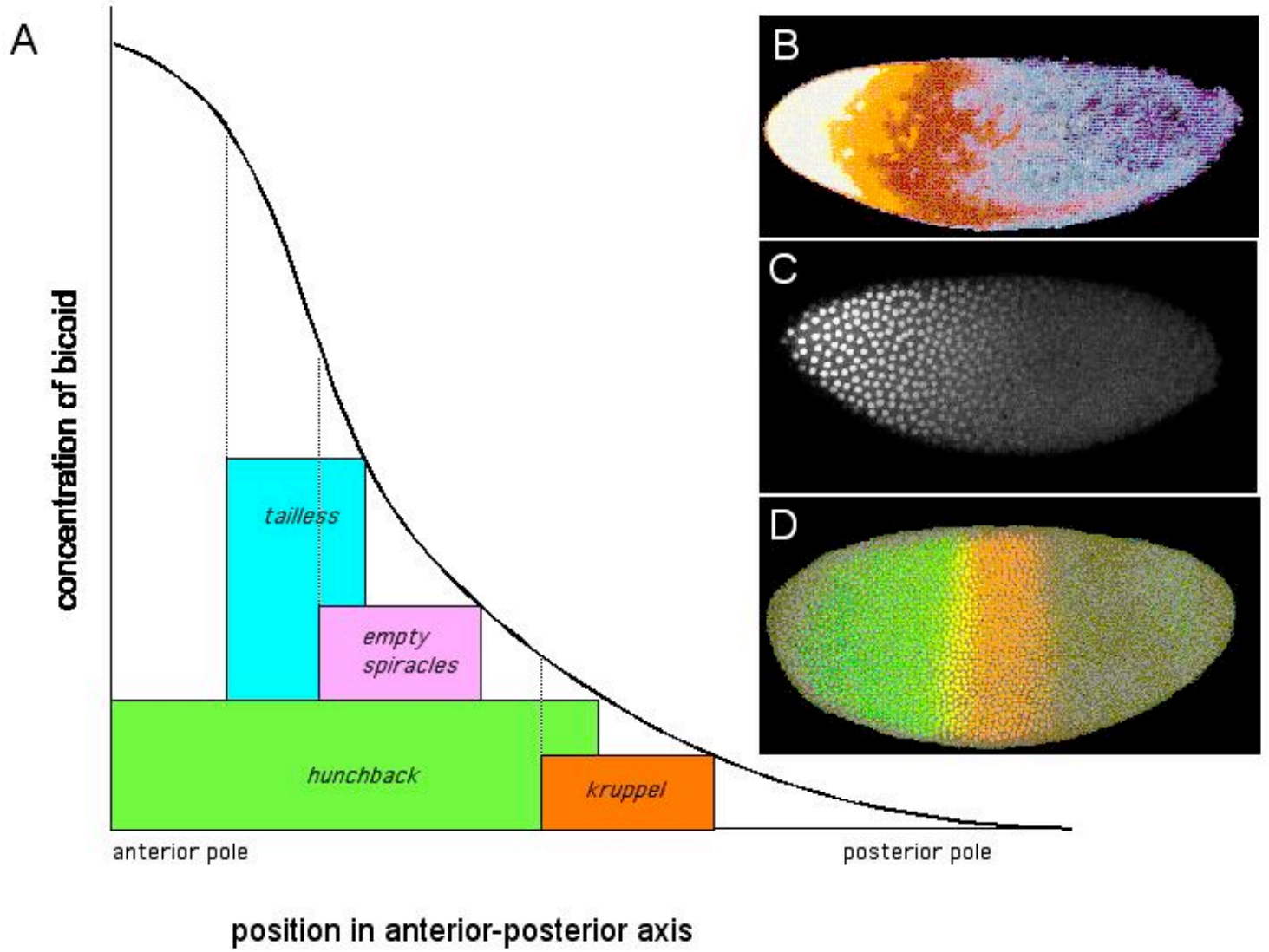
Bicoid positively regulates *hunchback* transcription





- Bicoid protein is a transcription factor, binds to cis-regulatory sequences (enhancer) of the *hunchback* gene
- *Hunchback* mRNA is produced above a certain Bicoid threshold, resulting in a distribution of *hunchback* that is steeply graded, with a sharp boundary

Bicoid also activates transcription of different downstream genes at different concentrations



bicoid mRNA

bicoid protein

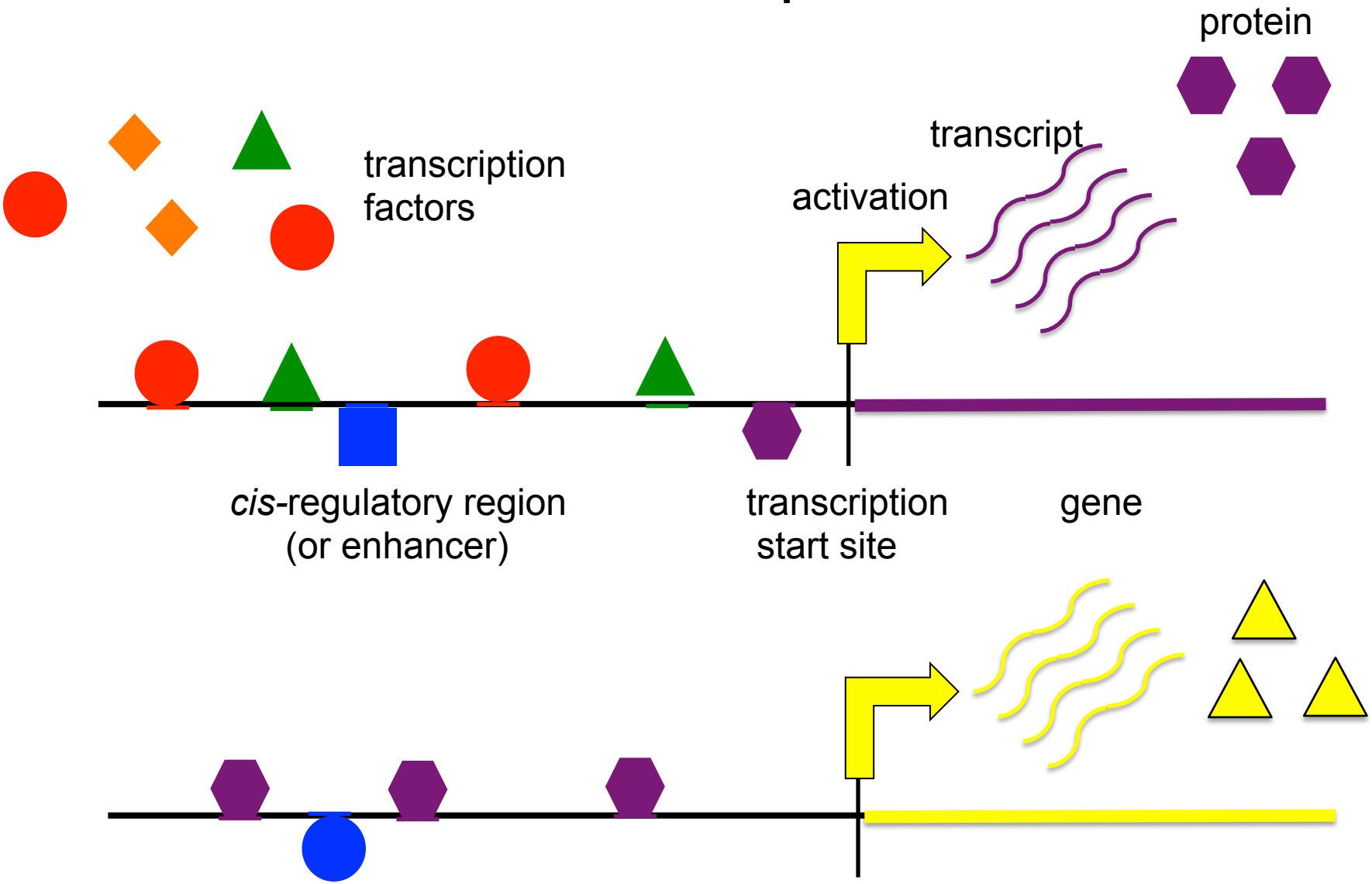
hb in green
Kr in orange

http://biology.kenyon.edu/courses/biol114/Chap11/Chapter_11.html

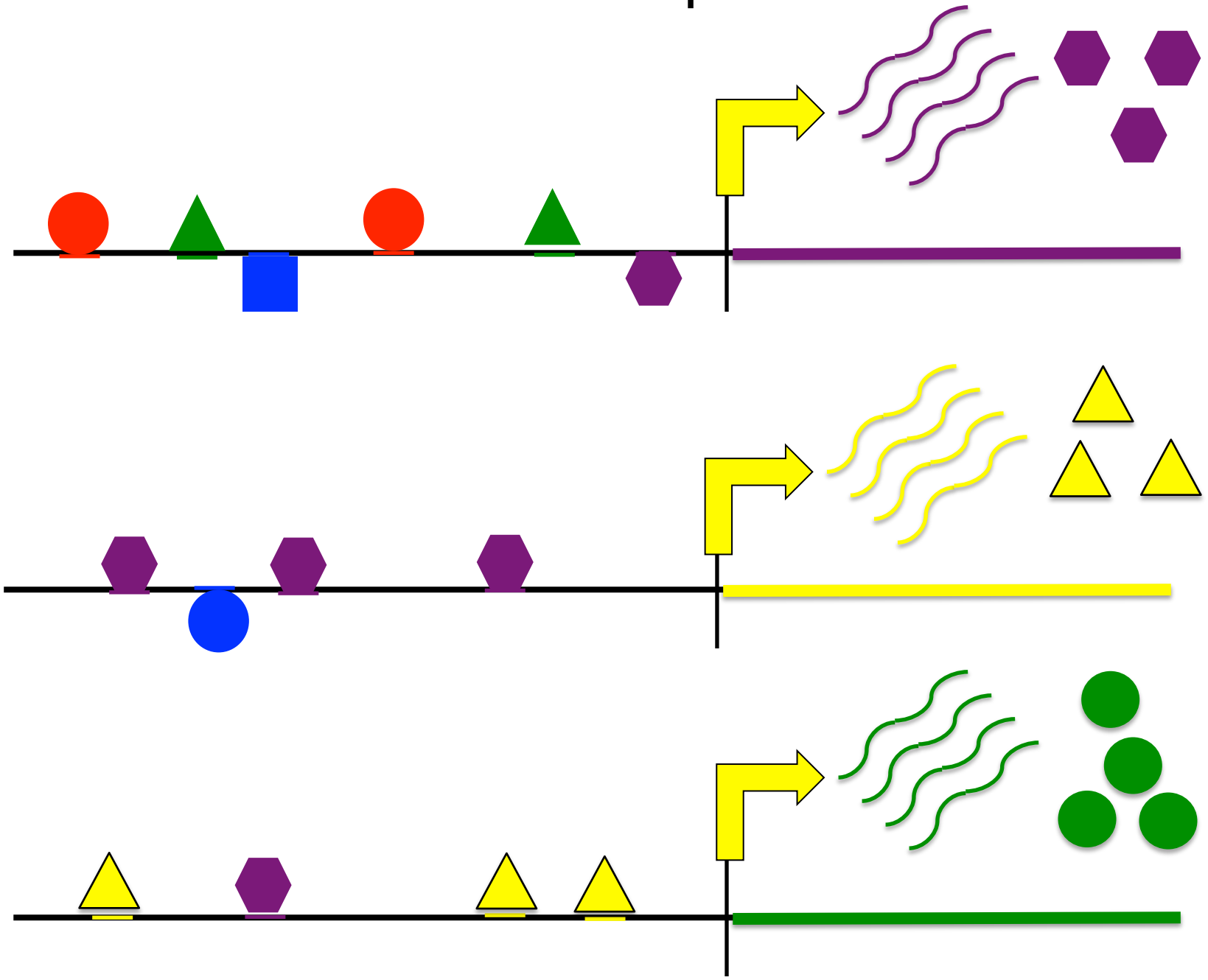
segmentation is a hierarchical transcriptional network

- earlier genes encode transcription factors that bind to regulatory regions of later genes and affect their transcription
- these later genes affect transcription of even later genes, and so on…
- these regulatory relationships can be activating or repressing
- these regulatory relationships are happening in space (i.e. determined by local concentrations of transcription factors in particular parts of the embryo)

hierarchical transcription network



hierarchical transcription network



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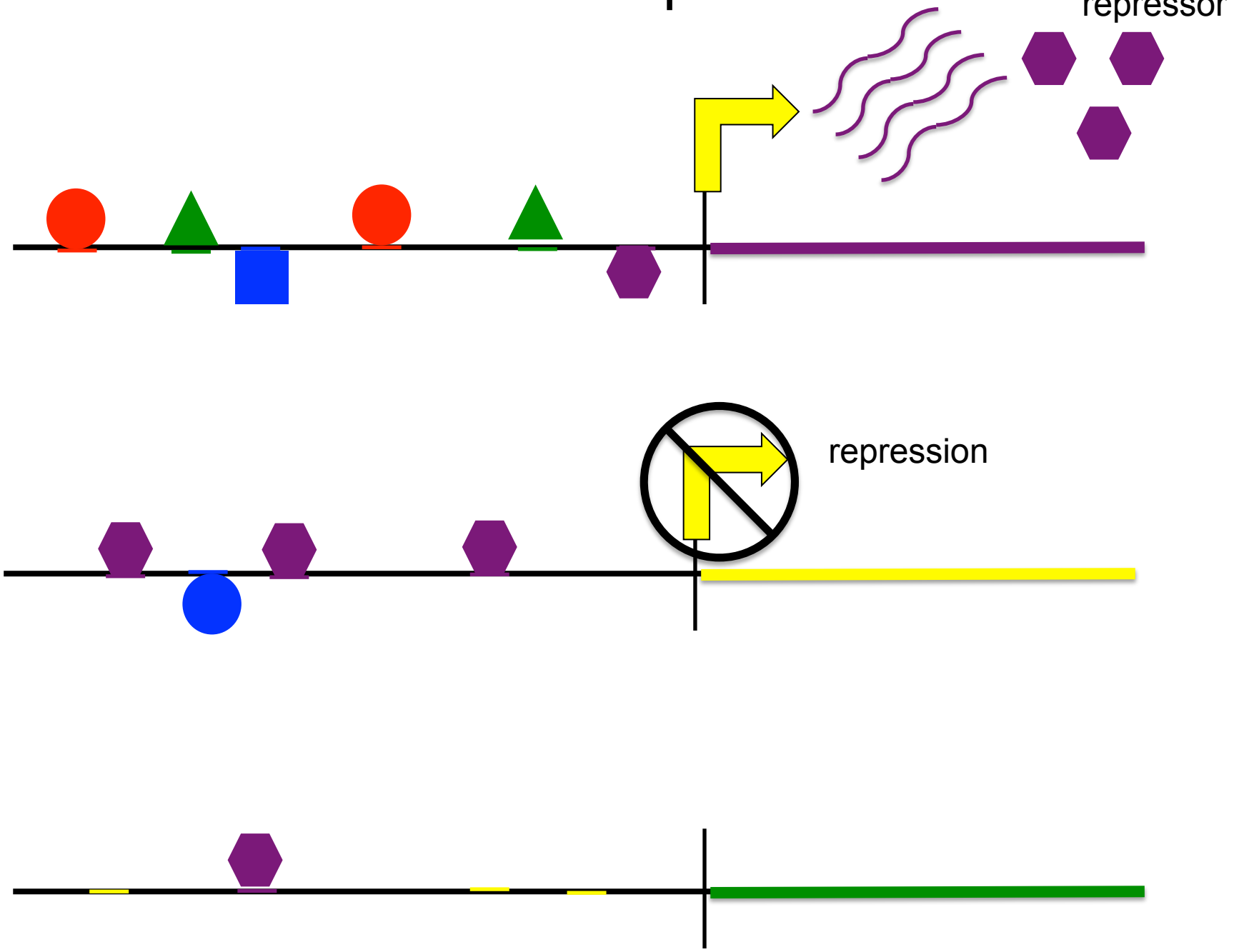

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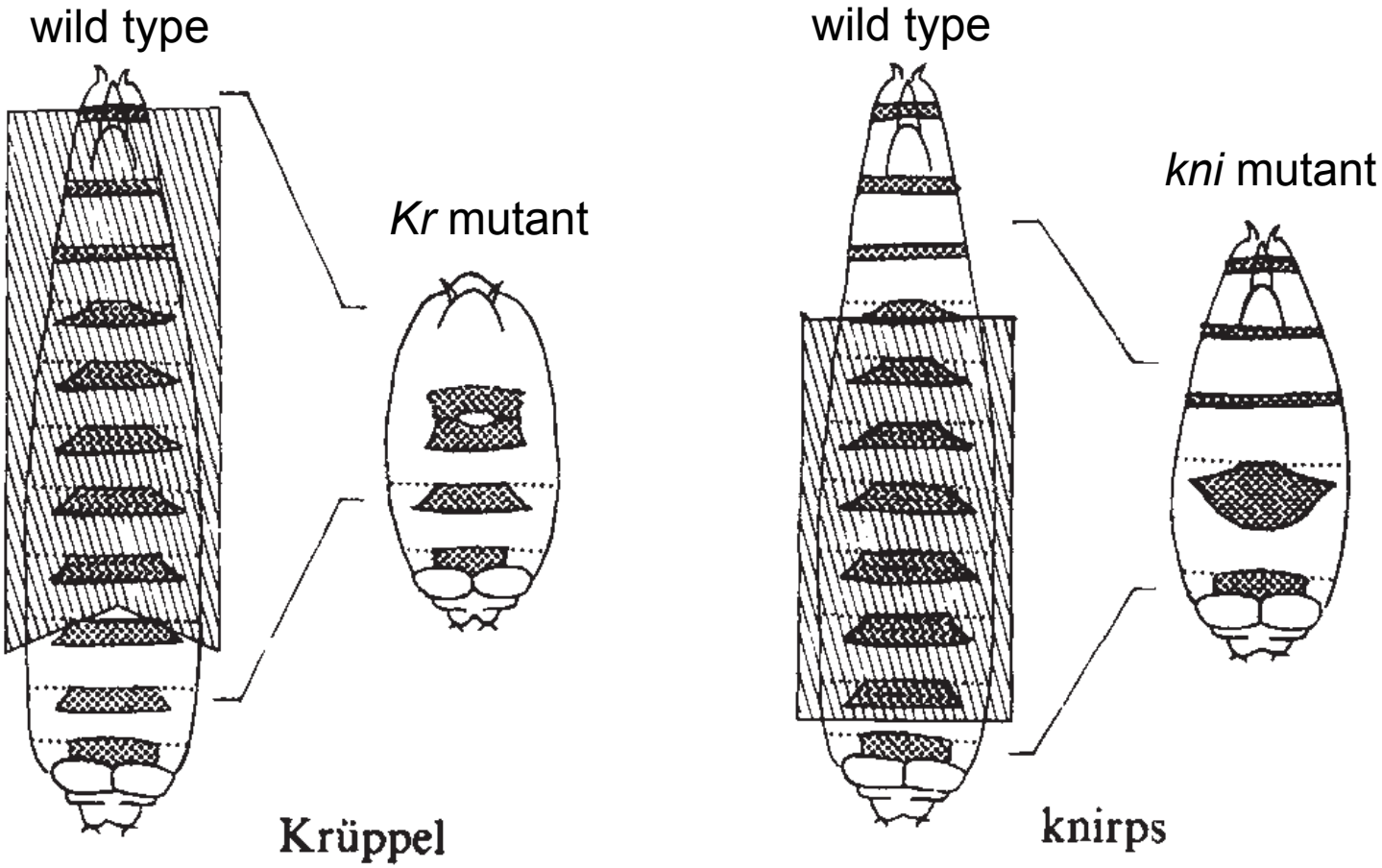
Maternal		establish gradients from anterior to posterior poles of eggs
Gap		define broad regions in the egg
Pair-rule		define 7 segments
Segment polarity		define 14 segments
Homeotic		define identity of each segment, what adult structures will form

gap gene mutant larvae have a continuous stretch of segments deleted



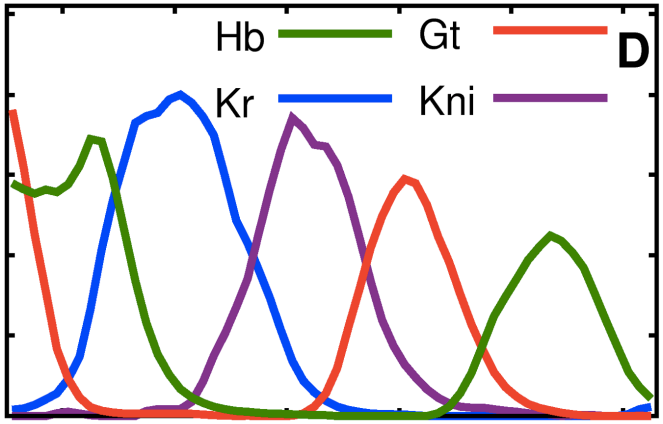
shaded areas correspond to the areas absent in mutant embryos

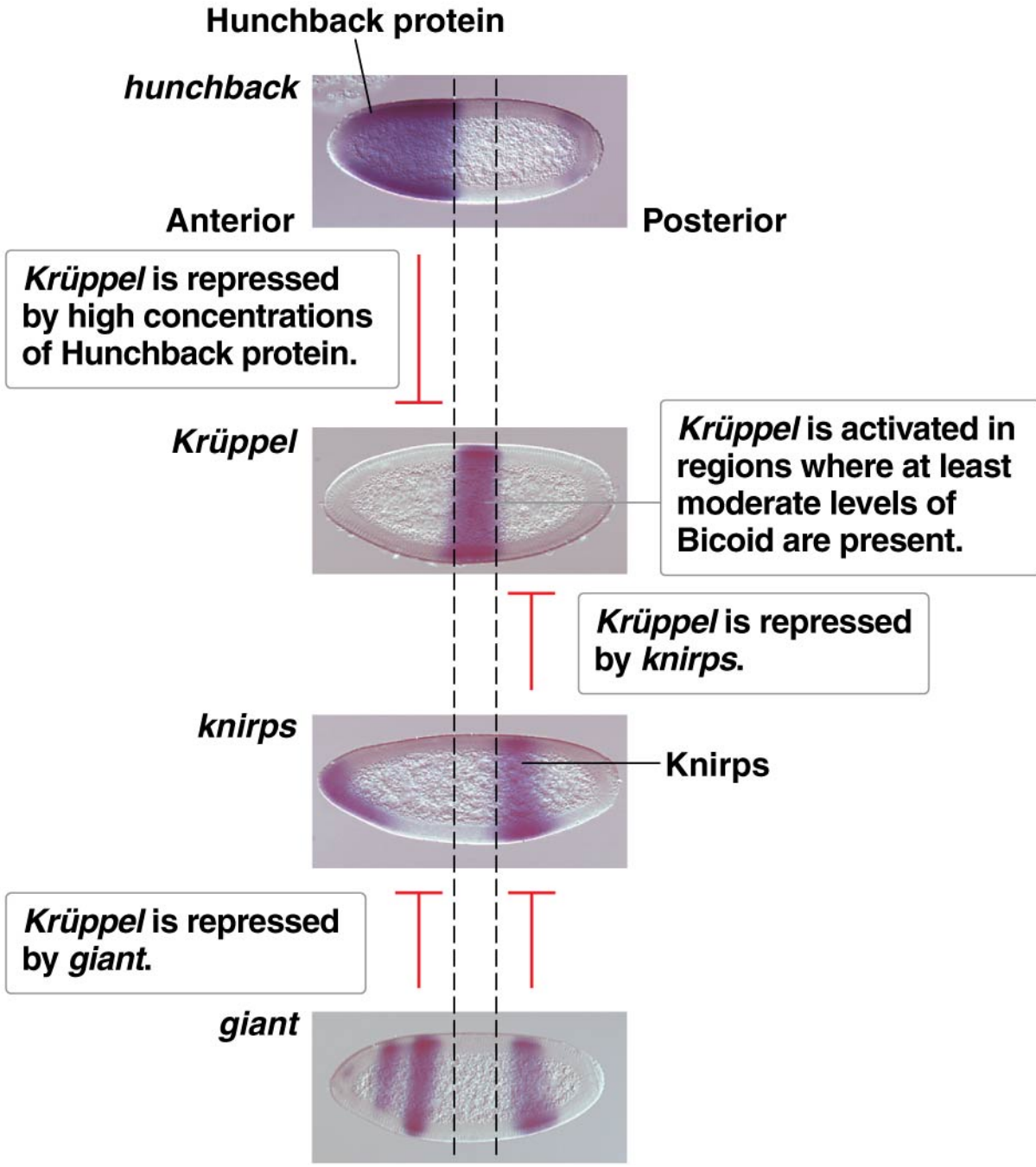
Nusslein-Volhard & Wieshaus, 1980

gap genes

- are expressed in broad domains
- are regulated by maternal genes
- regulate each other
- will regulate pair-rule genes

gap gene expression across the embryo





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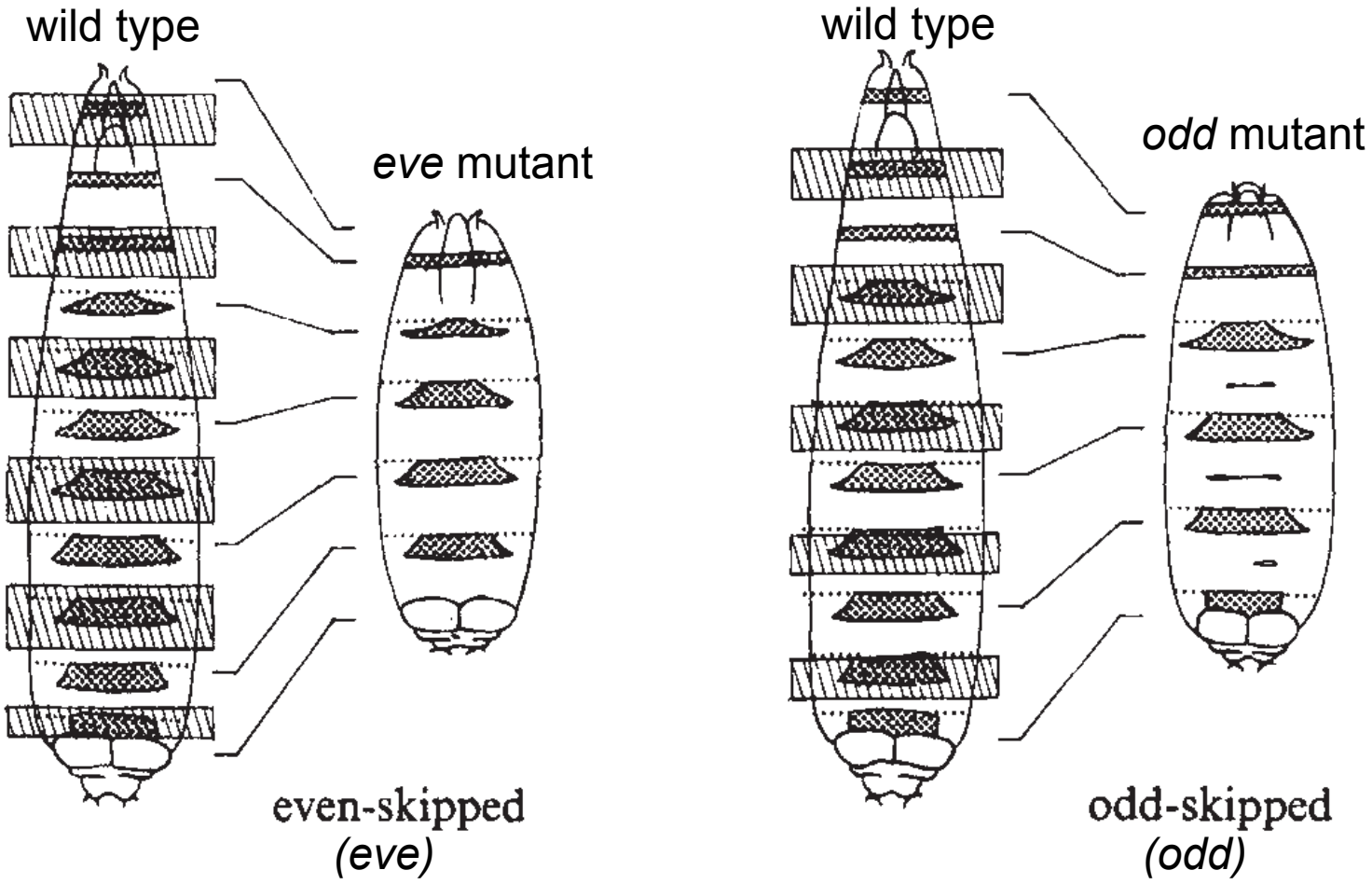
pair-rule gene mutant larvae have parts of alternating segments deleted

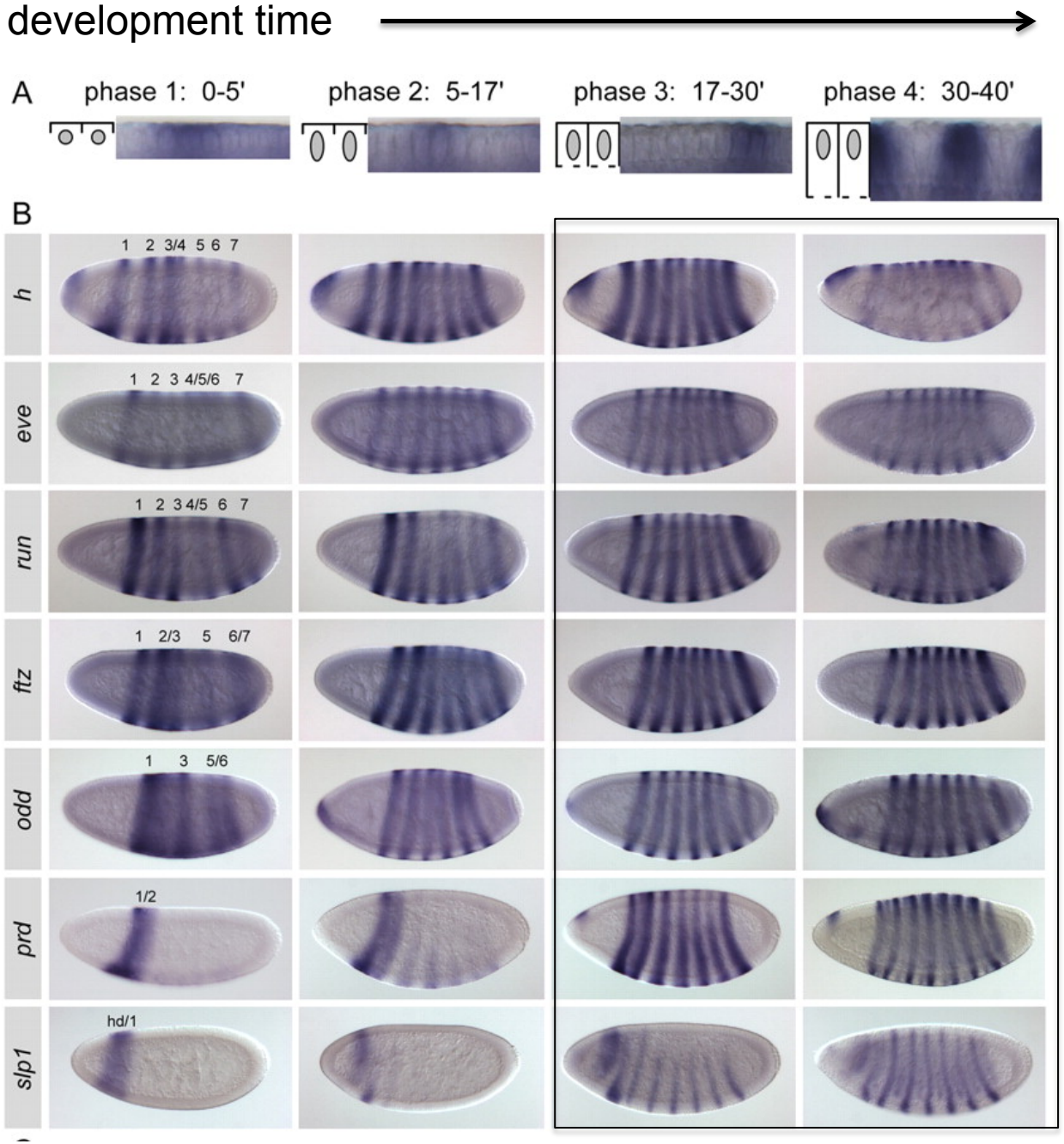


shaded areas correspond to the areas absent in mutant embryos

Nusslein-Volhard & Wieshaus, 1980

pair-rule genes

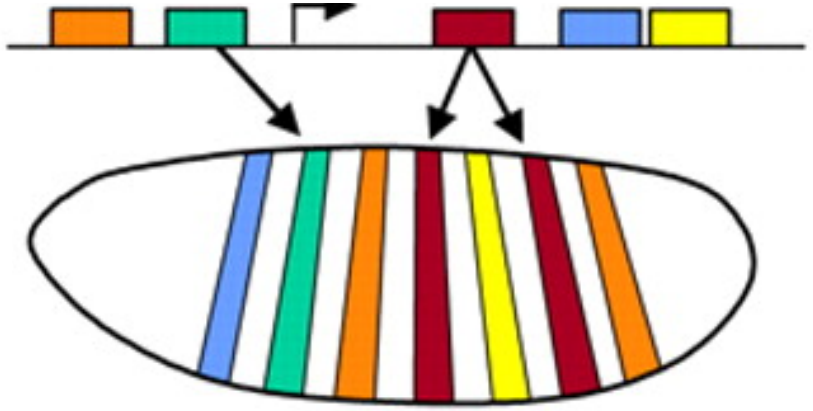
- are expressed in a pattern of 7 stripes
- are regulated by maternal genes and gap genes
- regulate each other
- will regulate segment polarity genes



Schroeder M D et al, (2011) *Development*

how are pair-rule genes regulated?

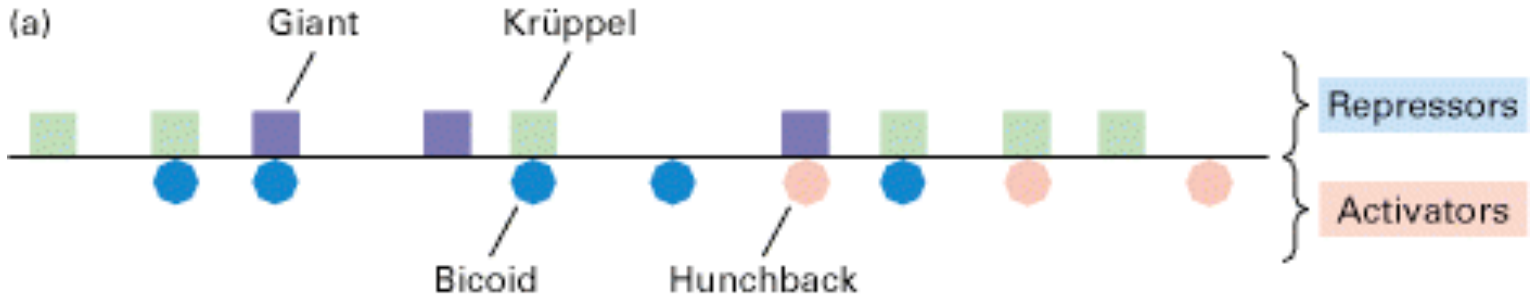
stripe specific *cis*-regulatory elements (enhancers)



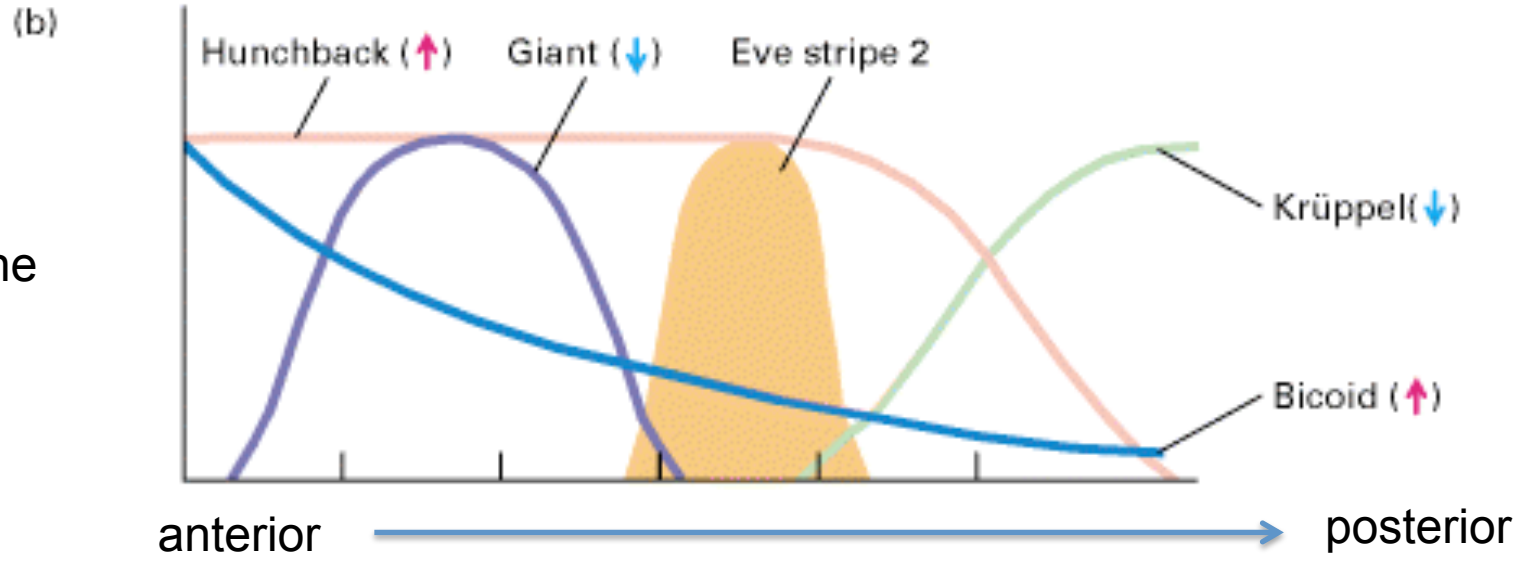
- **each stripe** of a pair rule gene may **have its own enhancer** element
- these **enhancers** will bind the **transcription factors** (products of gap and maternal genes) that are present in that portion of the embryo

activator and repressor TFs bind to enhancers to turn on expression of pair-rule gene in a narrow stripe

eve stripe 2 enhancer



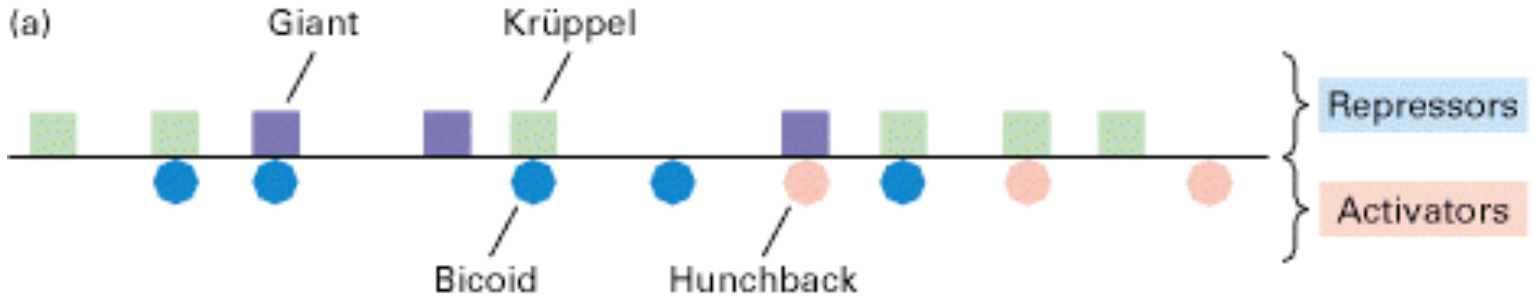
positions of TFs along the embryo



Lodish et al, *Molecular Cell Biology*

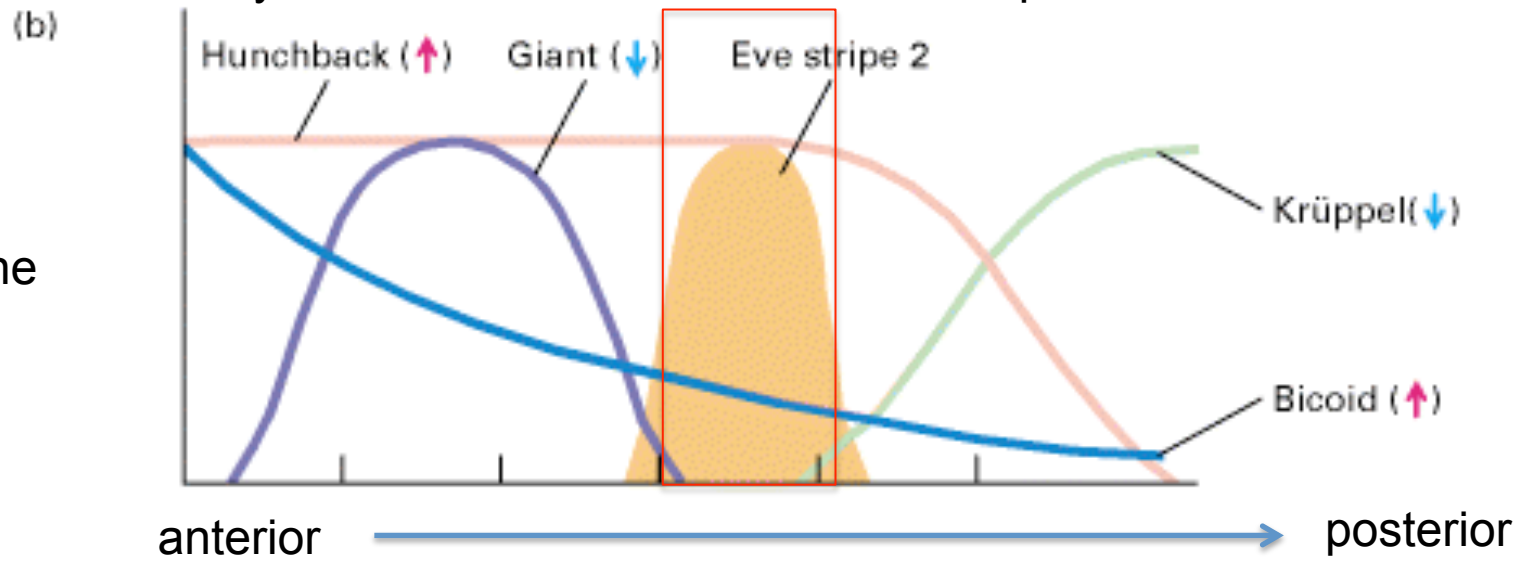
activator and repressor TFs bind to enhancers to turn on expression of pair-rule gene in a narrow stripe

eve stripe 2 enhancer



eve is transcribed only where activators are bound, but repressors are not

positions of TFs along the embryo



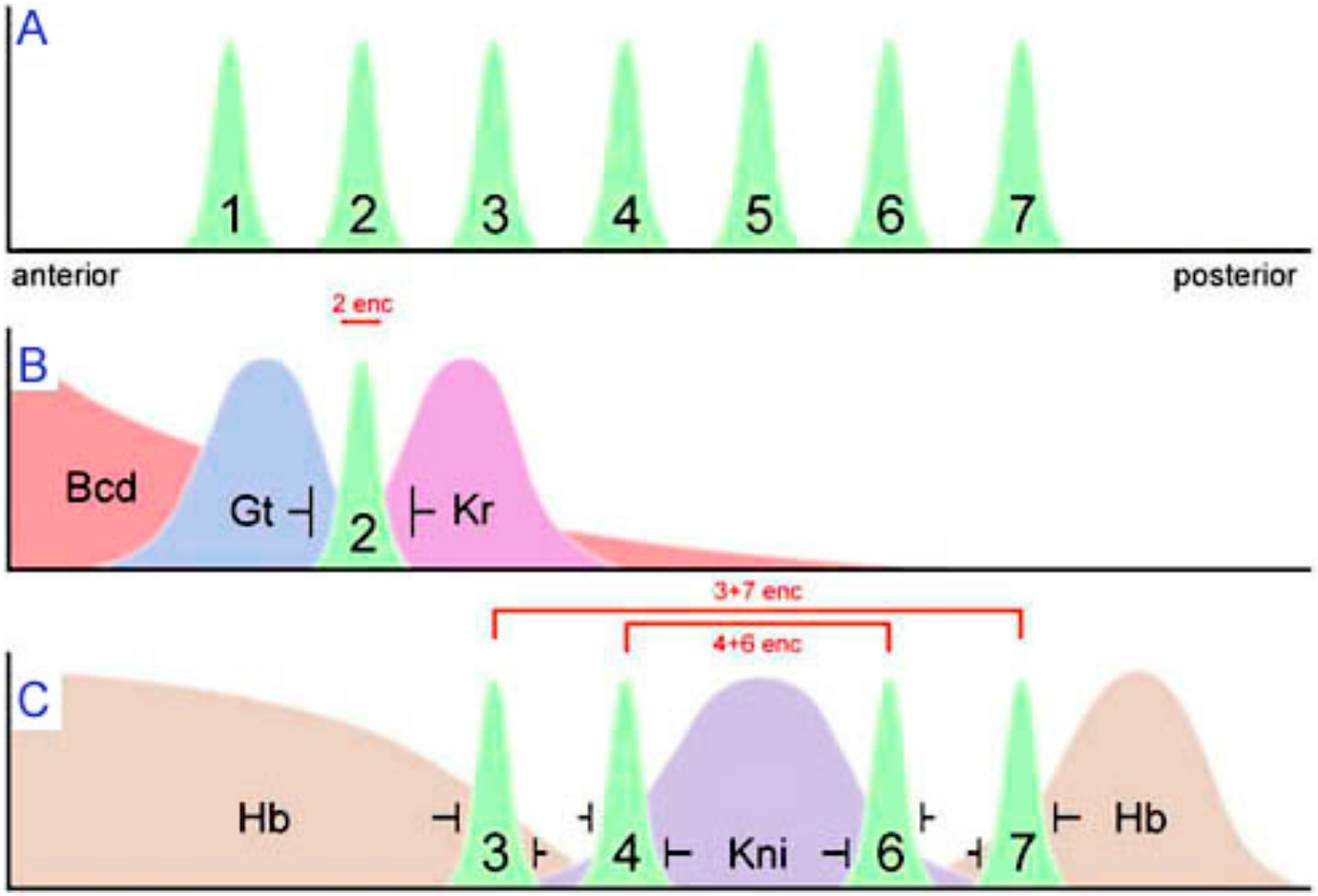
Lodish et al, *Molecular Cell Biology*

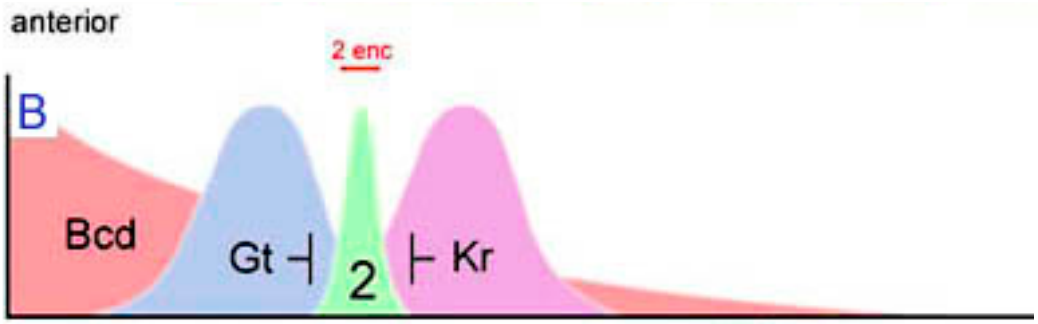
transcription of *eve* gene in stripey pattern at particular places in the embryo determined by TFs

all 7 *eve* stripes, most have unique enhancer, bind different combinations of TFs

stripe 2 regulation: activated by Bcd repressed by Gt and Kr

Stripe 3,4,6,7: repressed by Hb, Kni

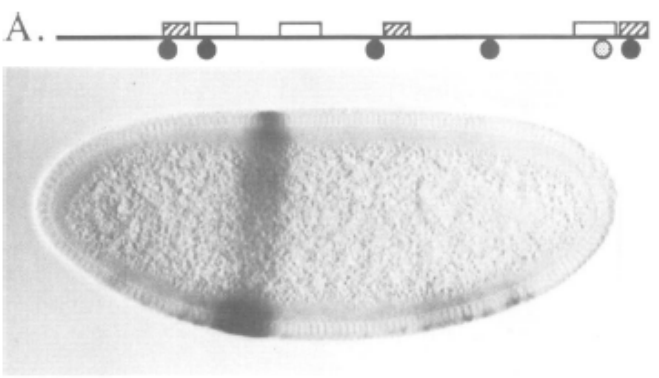




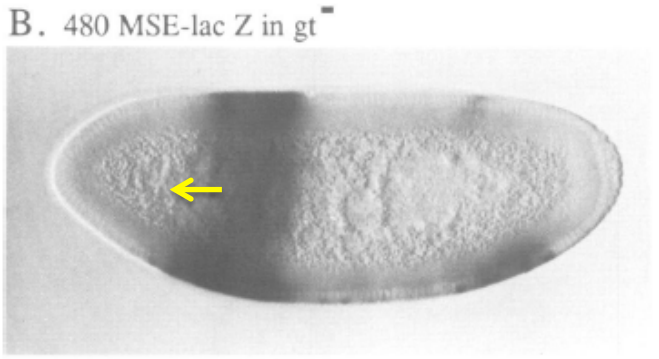
eve stripe 2

eve stripe 2 expression

wild-type



wild-type enhancer

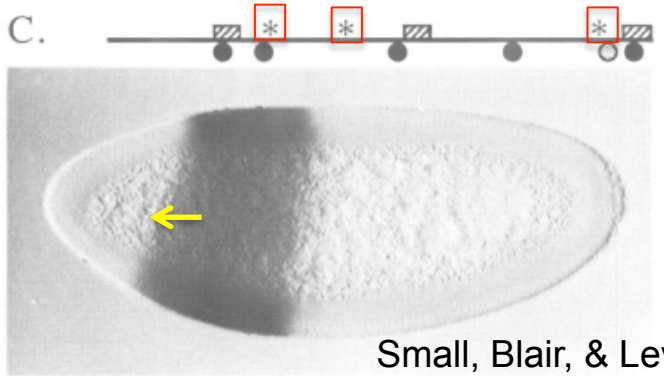


gt- mutant

no GT protein in embryo

anterior expansion of eve stripe

same phenotype!



Gt binding sites mutated

no GT binding sites in stripe 2 enhancer

Small, Blair, & Levine (1992)

what have we learned about regulation of gene expression from these studies?

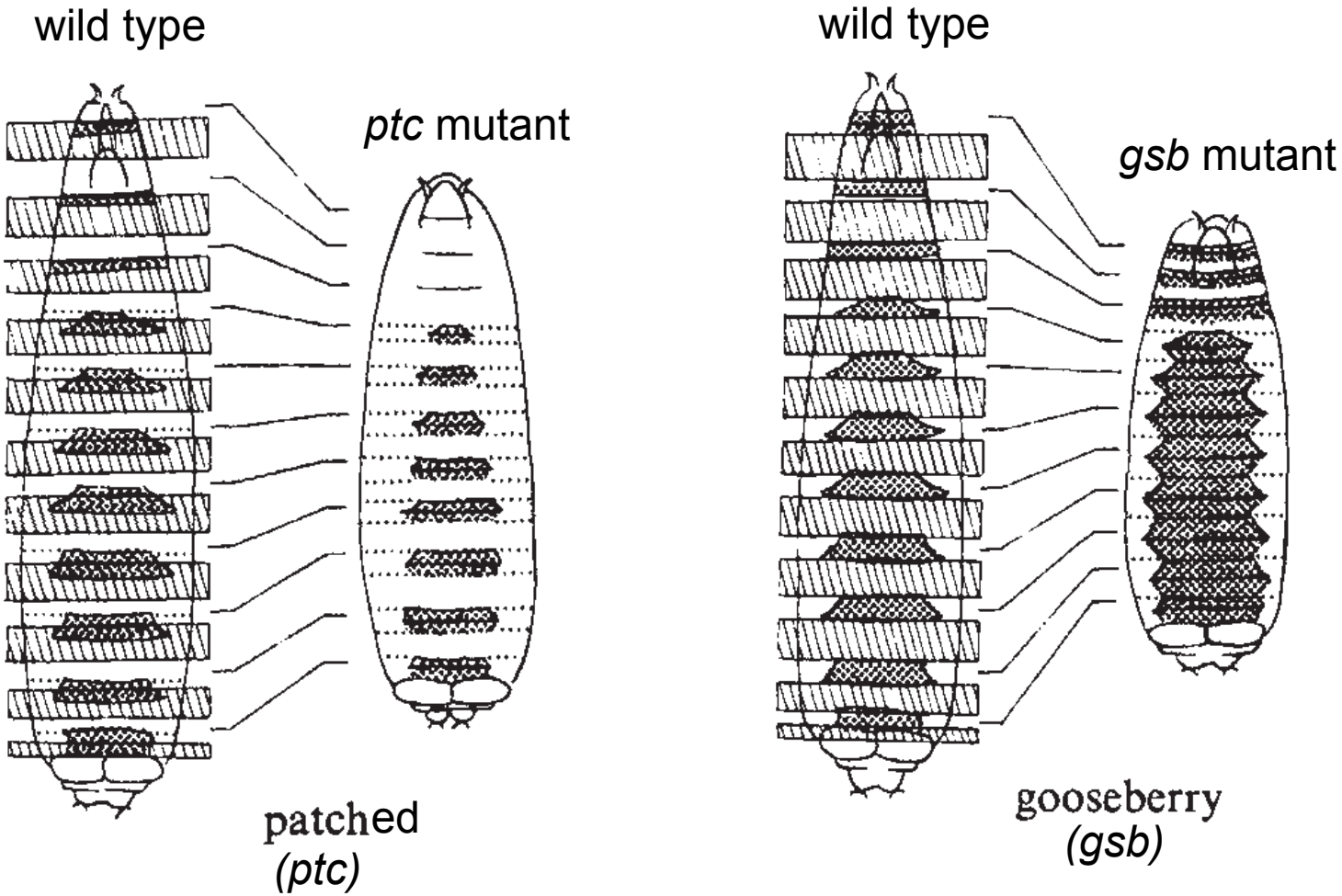
- concentration of **transcription factors** in time and space can control transcription of downstream genes by binding to their **enhancers**

- different enhancers can control different places where genes are expressed

- studies of *eve* stripe 2 enhancer have been a model for discovering many “rules” about how enhancers and transcription factors work!

gene category	expression pattern (of one gene in category)	function
Maternal		establish gradients from anterior to posterior poles of eggs
Gap		define broad regions in the egg
Pair-rule		define 7 segments
Segment polarity		define 14 segments
Homeotic		define identity of each segment, what adult structures will form

segment polarity mutants have parts of each segment deleted



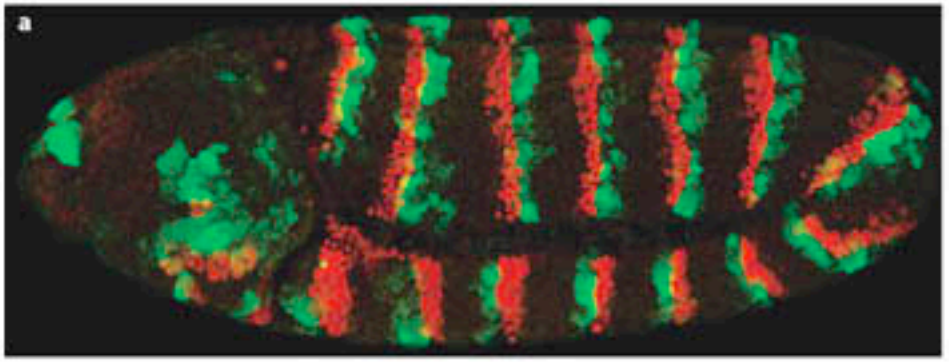
shaded areas correspond to the areas absent in mutant embryos

Nusslein-Volhard & Wieshaus, 1980

segment polarity genes

- are expressed in a pattern of 14 stripes
- are regulated by pair-rule genes
- regulate each other

showing expression of two segment polarity genes,
wg in green
en in red



Tomlin & Axelrod (2007)

gene category	expression pattern (of one gene in category)	function
Maternal		establish gradients from anterior to posterior poles of eggs
Gap		define broad regions in the egg
Pair-rule		define 7 segments
Segment polarity		define 14 segments
Homeotic/ Hox		define identity of each segment, what adult structures will form

--summary--

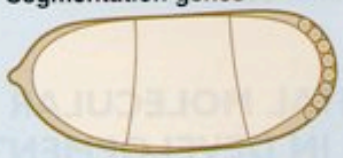
Genetic hierarchy	Functions	Representative genes	Effects of mutation
Maternal-effect genes 	Establish gradients from anterior and posterior poles of the egg	Bicoid Swallow Oskar Caudal Torso Trunk	Major disturbances in anteroposterior organization
Segmentation genes  *Gap genes*	Define broad regions in the egg	Empty spiracles Hunchback Krüppel Knirps Orthodenticle Tailless	Adjacent segments missing in a major region of the body
 *Pair-rule genes*	Define 7 segments	Hairy Even skipped Runt Fushi tarazu Odd paired Odd skipped Paired	Part of pattern deleted in every other segment
 *Segment-polarity genes*	Define 14 segments	Engrailed Gooseberry Hedgehog Patched Smoothened Wingless	Segments replaced by their mirror images
Homeotic genes 	Determine regional characteristics	Antennapedia complex Bithorax complex	Inappropriate structures form for a given segmental level

FIGURE 4-1 Sequence of genetic control of early development in *Drosophila*. Within each level of genetic control are listed representative genes.

expression pattern

gradient from head to tail or tail to head

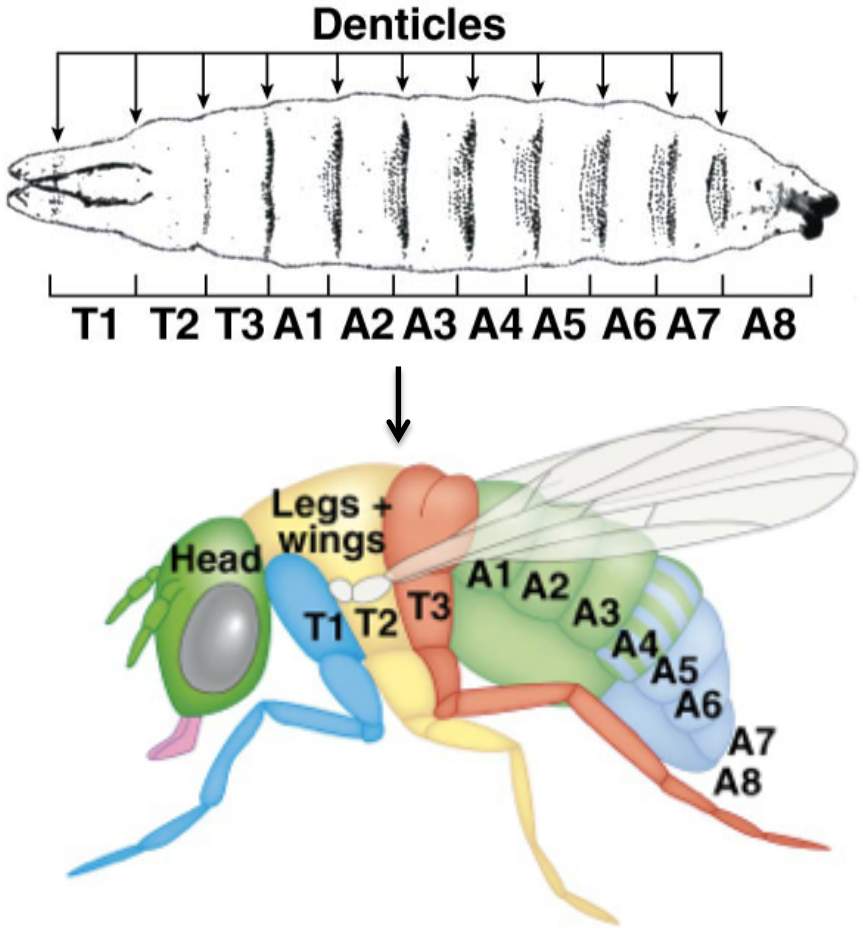
broad expression domains (1-3 wide bands)

seven stripes

fourteen stripes

variable, see other info on hox genes!

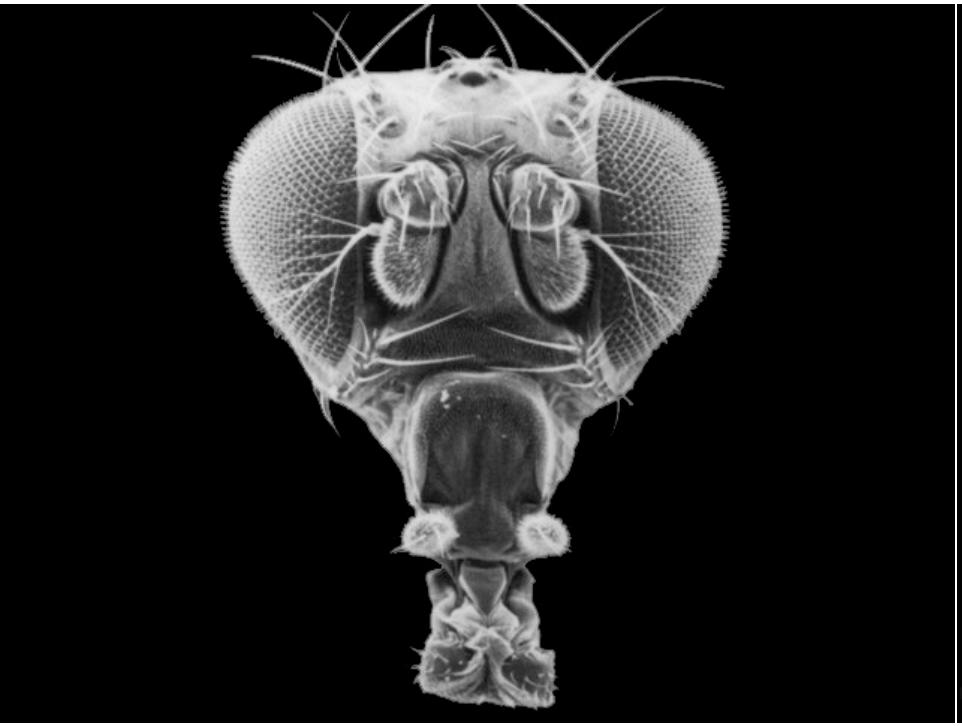
how do we go from repeating segments to distinct segmental identities?



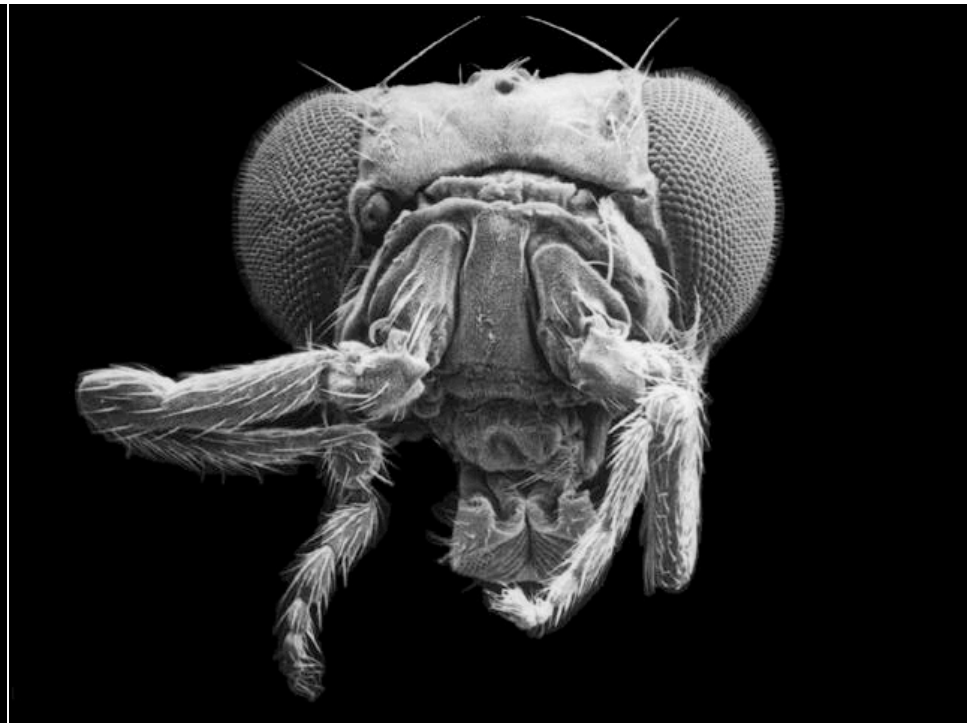
hox genes!

- The **14 segments** of the embryo acquire their **unique identities** through the action of the **Hox genes**
- these genes code for **transcription factors**, that **regulate** many downstream genes
- different regions along the embryo express **different combinations of HOX genes**, and these combinations define different positional identities (leading to the development of different organs and cell types)
- they contain a DNA-binding domain known as a **homeobox** (hox genes are a subset of homeobox genes)
- mutant hox genes can cause **homeotic phenotypes** (hox genes are a subset of homeotic genes)

Homeosis is a replacement of a body part with another, apparently normal body part (W. Bateson, 1894)



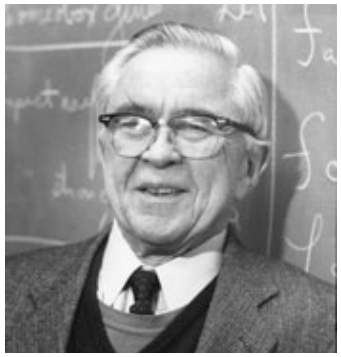
wild-type



antennapedia mutant: Antenna are transformed into metathoracic (second second thoracic segment) legs.

image from Dr. Rudi Turner, via Interactive Fly

hox genes in flies

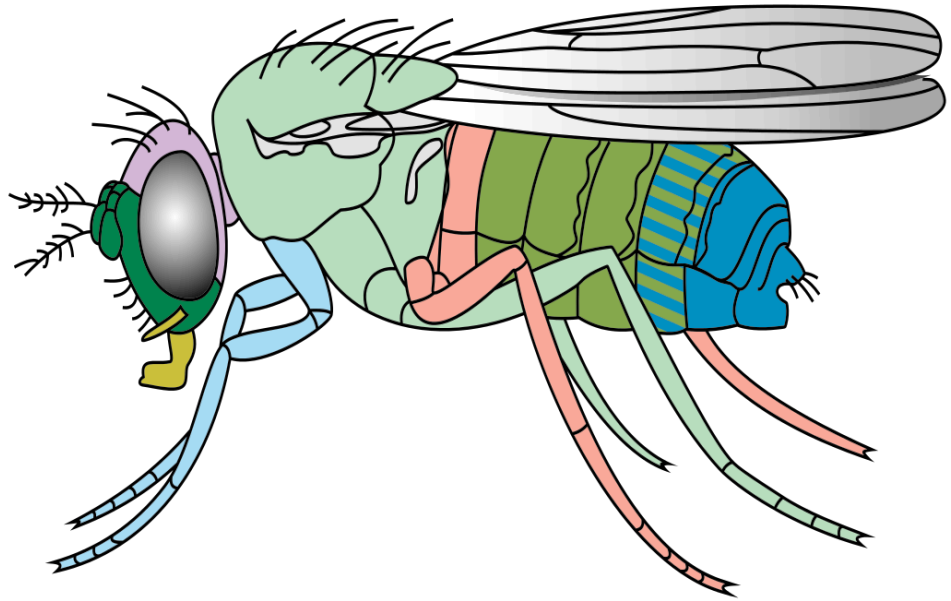


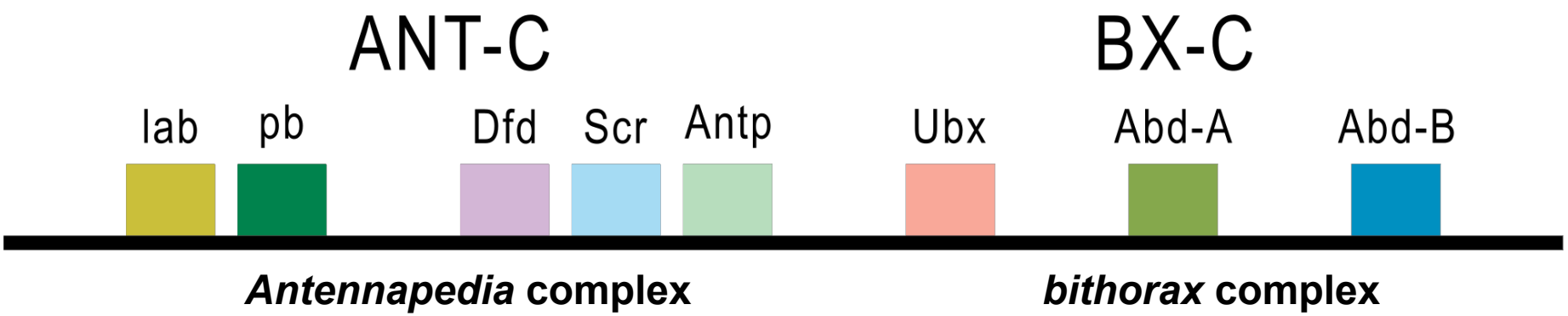
- Ed Lewis systematically worked out the structure of hox genes in flies in the 1950's

- The five genes of the ***Antennapedia*** **complex** and the three genes of the ***bithorax*** **complex** are both found on chromosome 3 of *Drosophila*

- The order of the genes in the clusters reflects their order of expression, from anterior to posterior

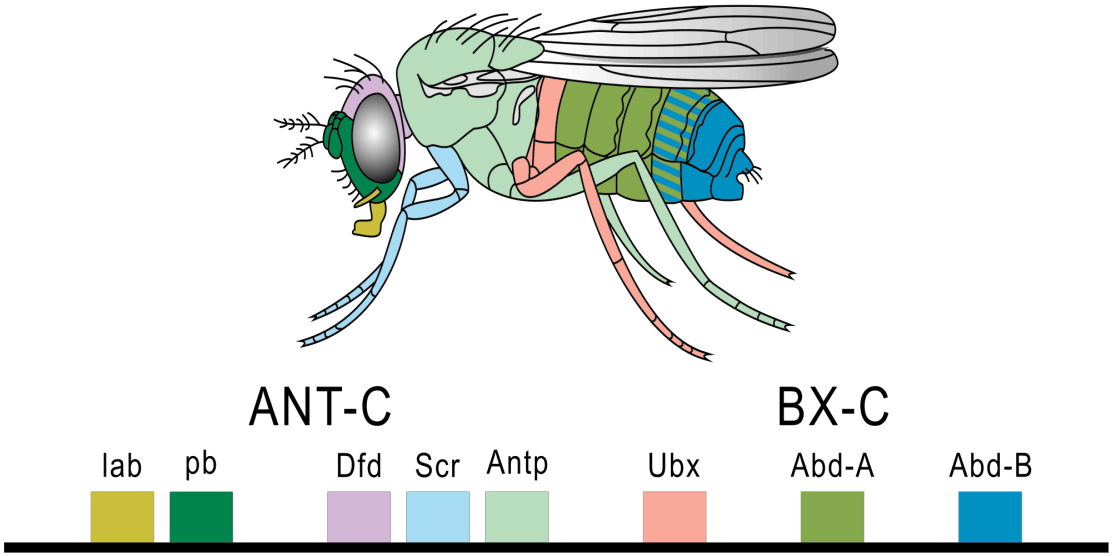
hox genes are expressed in the anterior-posterior axis in the order they are on the chromosome





wild-type flies have only one pair of wings!

UBX is responsible.



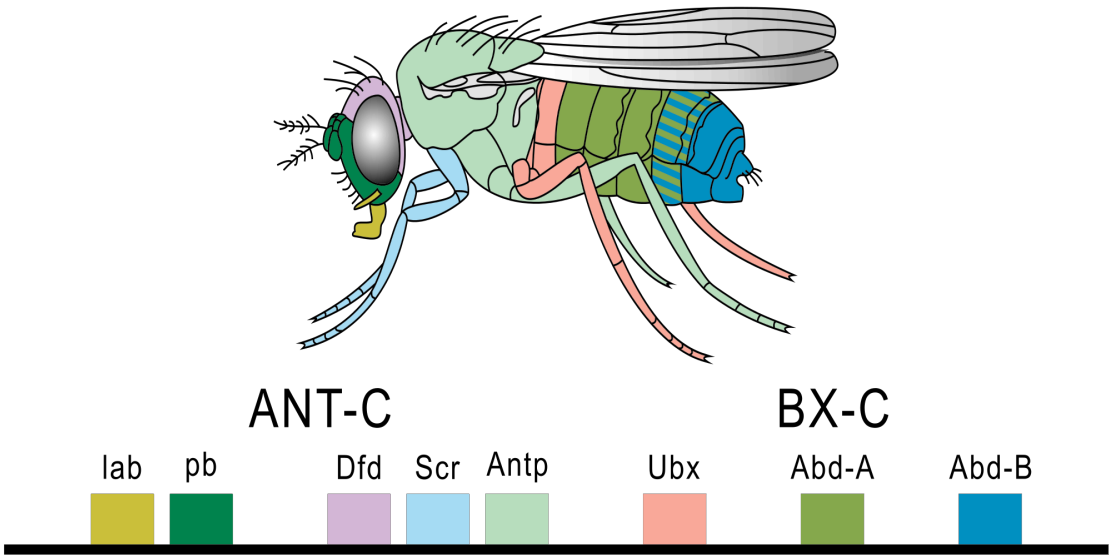
wild-type

ubx mutant

E. Lewis

wild-type flies have only one pair of wings!

UBX is responsible.



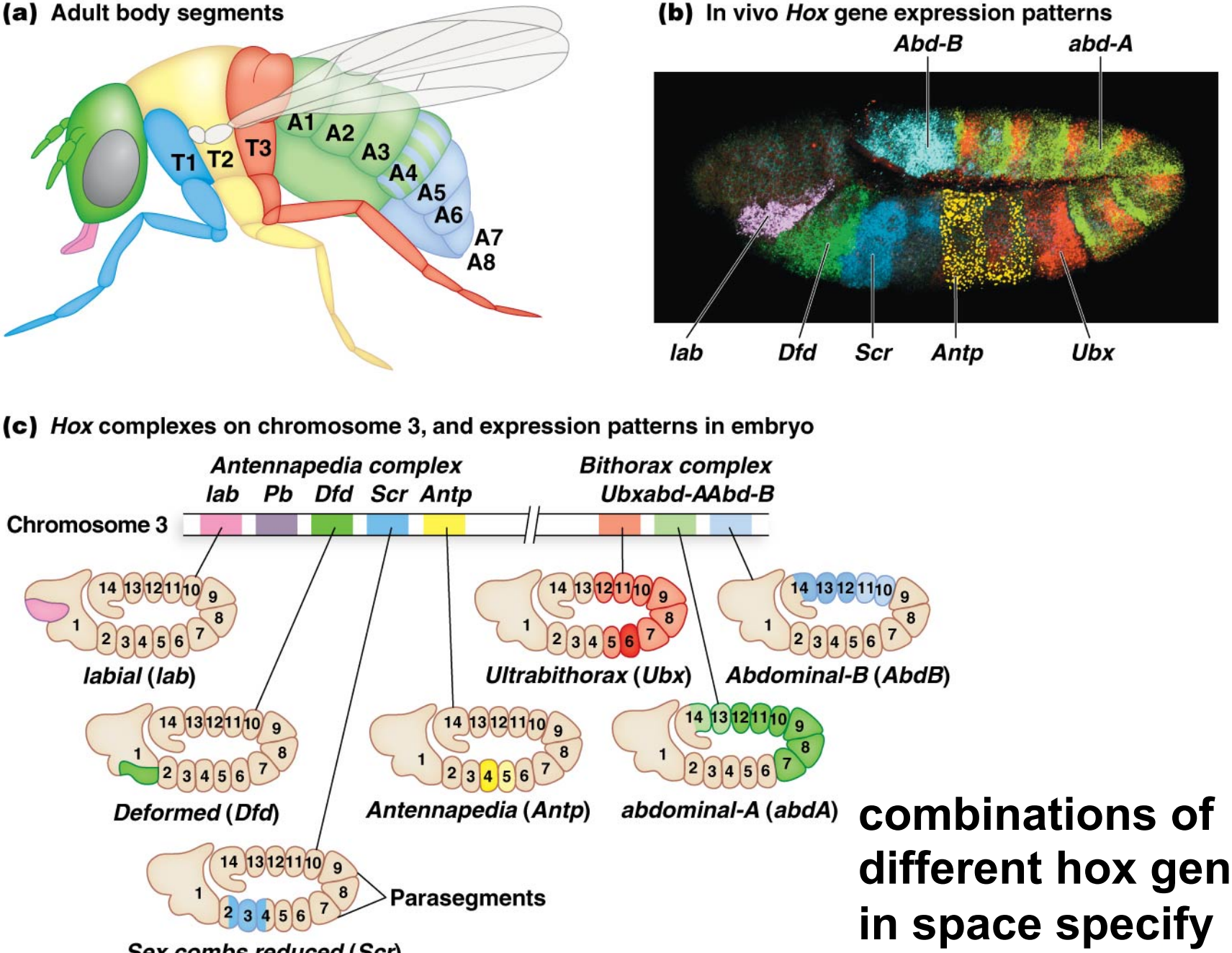
If *ubx* is non-functional, then the pink T3 segment above (normally wingless)

is instead assigned the mint green function of the T2 segment (which has wings)

this essentially duplicates the T2 segment where T3 should be

ubx mutant

E. Lewis



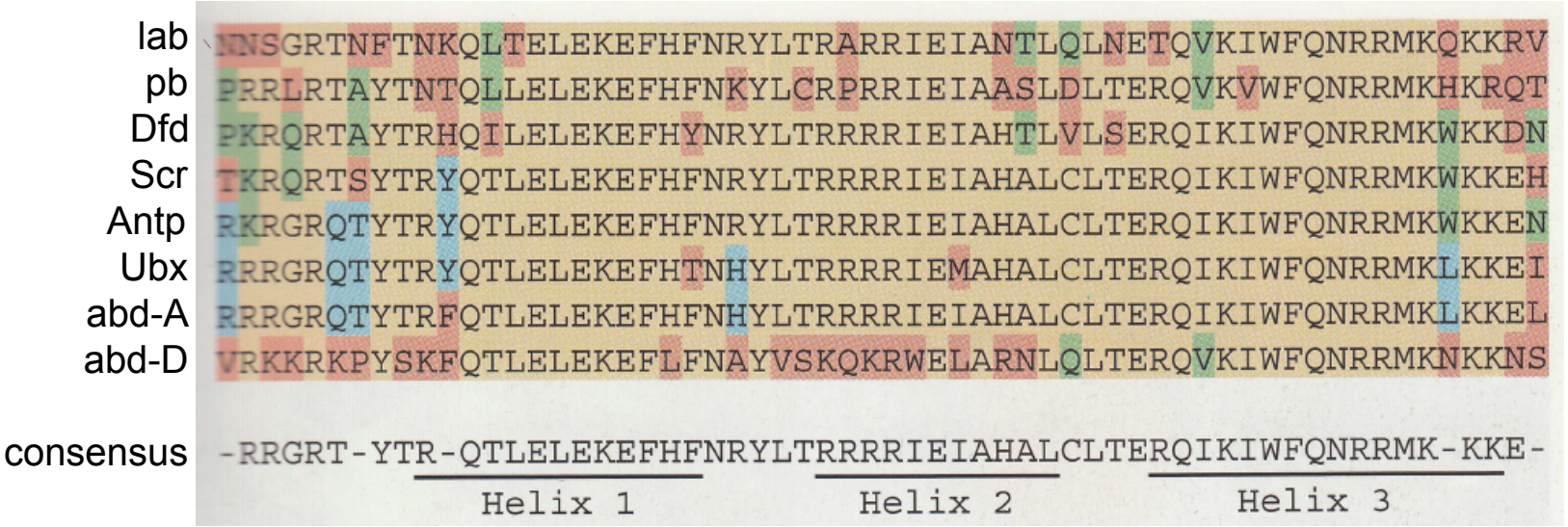
combinations of different hox genes in space specify segment identity

Hox genes have homeobox domains

(and are a subset of homeodomain-containing TFs)

The homeobox is a highly conserved DNA-binding domain

alignment of homeodomains of *Drosophila* homeotic genes



Carroll et al, *DNA to Diversity*, 2005

Developmental genetics

- development and cell differentiation
- positional information
- *Drosophila* model
- Hox genes and the conserved developmental toolkit

Cell, Vol. 37, 409–414, June 1984, Copyright © 1984 by MIT 0092-8674/84/060409-06 $02.00/0

Cloning of an X. laevis Gene Expressed during Early Embryogenesis Coding for a Peptide Region Homologous to Drosophila Homeotic Genes

Andrés E. Carrasco, William McGinnis, Walter J. Gehring, and Eddy M. De Robertis
Department of Cell Biology
Biozentrum, University of Basel
Klingelbergstr. 70
CH 4056 Basel, Switzerland

Cell, Vol. 38, 675–680, October 1984, Copyright © 1984 by MIT 0092-8674/84/100675-06 $02.00/0

Molecular Cloning and Chromosome Mapping of a Mouse DNA Sequence Homologous to Homeotic Genes of Drosophila

William McGinnis,*† Charles P. Hart,‡ Walter J. Gehring,* and Frank H. Ruddle‡
*Department of Cell Biology
Biocenter
University of Basel
Klingelbergstrasse 70
CH-4056 Basel, Switzerland
‡Department of Biology
Yale University
New Haven, Connecticut 06511

Cell, Vol. 38, 667–673, October 1984, Copyright © 1984 by MIT

Human DNA Sequences Homologous to a Protein Coding Region Conserved between Homeotic Genes of Drosophila

Michael Levine,* Gerald M. Rubin, and Robert Tjian
Department of Biochemistry
University of California
Berkeley California 94720

the discovery of Hox genes in other animals quickly followed!

Drosophila Dfd/ Hox4B

Carroll et al, *DNA to Diversity*, 2005

what about organization and function of Hox genes in other animals?

just a few years later!

Cell, Vol. 57, 367–378, May 5, 1989, Copyright © 1989 by Cell Press

The Murine and Drosophila Homeobox Gene Complexes Have Common Features of Organization and Expression

Anthony Graham, Nancy Papalopulu, and Robb Krumlauf
Division of Eucaryotic Molecular Genetics
National Institute for Medical Research
The Ridgeway, Mill Hill
London NW7 1AA
England

The EMBO Journal vol.8 no.5 pp.1497 – 1505, 1989

The structural and functional organization of the murine HOX gene family resembles that of *Drosophila* homeotic genes

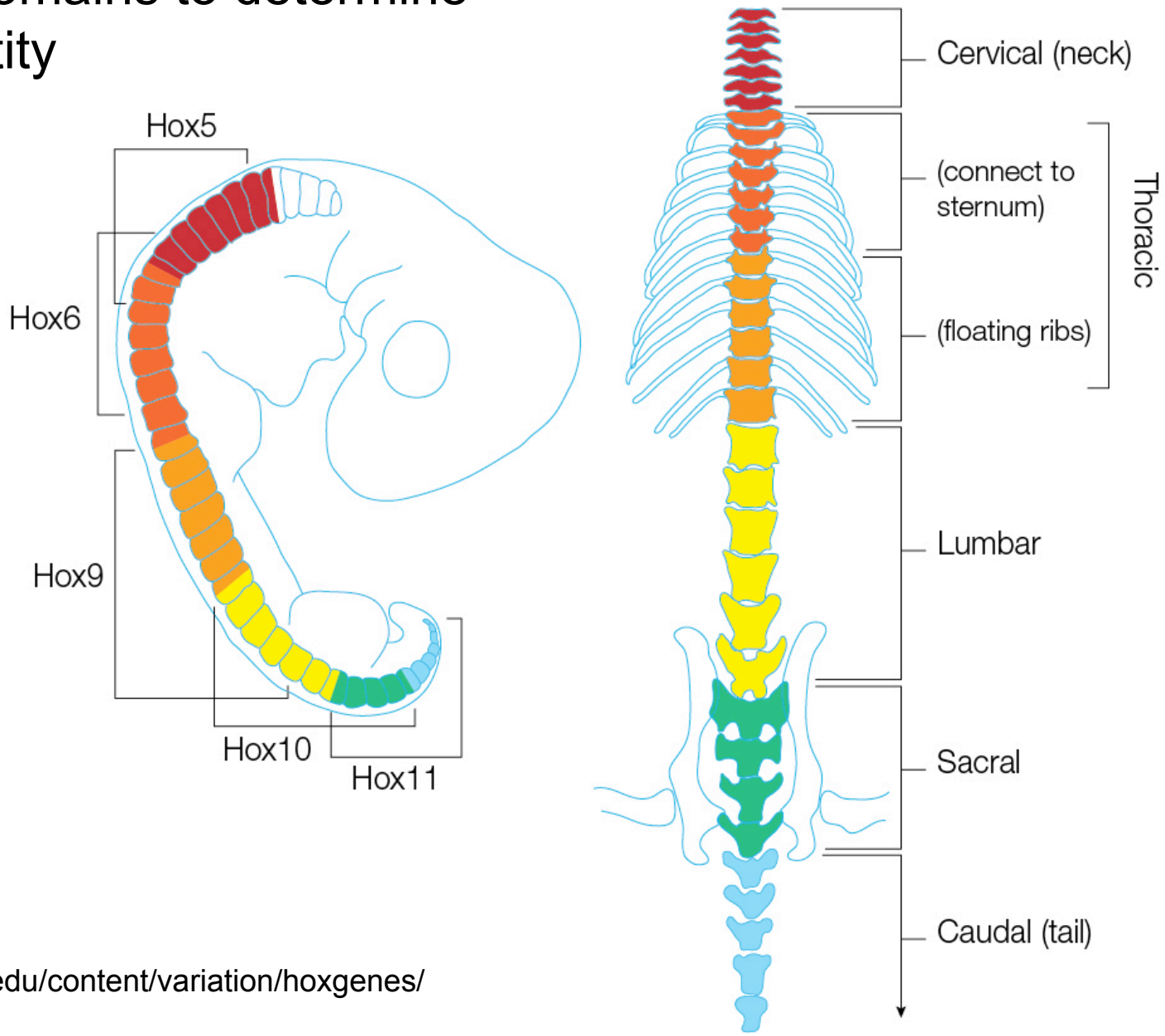
Denis Duboule[1] and Pascal Dollé

Laboratoire de génétique moléculaire des eucaryotes du CNRS, U.184 de biologie et de génie génétique de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg cédex, France

[1]Present address: EMBL, Meyerhofstraße 1, Postfach 10.22 09, D-6900 Heidelberg, FRG

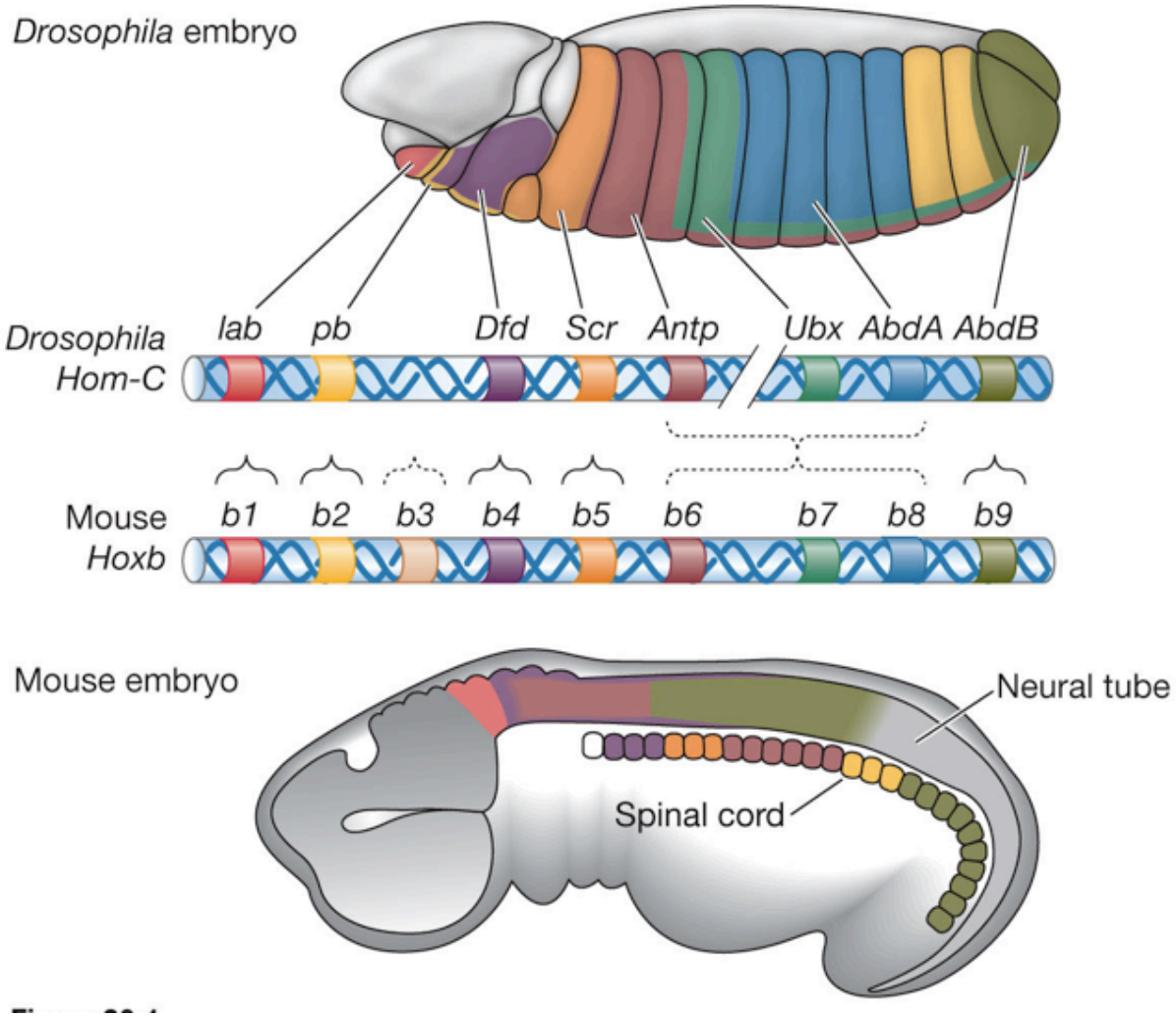
Communicated by P.Chambon

Hox genes are expressed in overlapping domains to determine segment identity

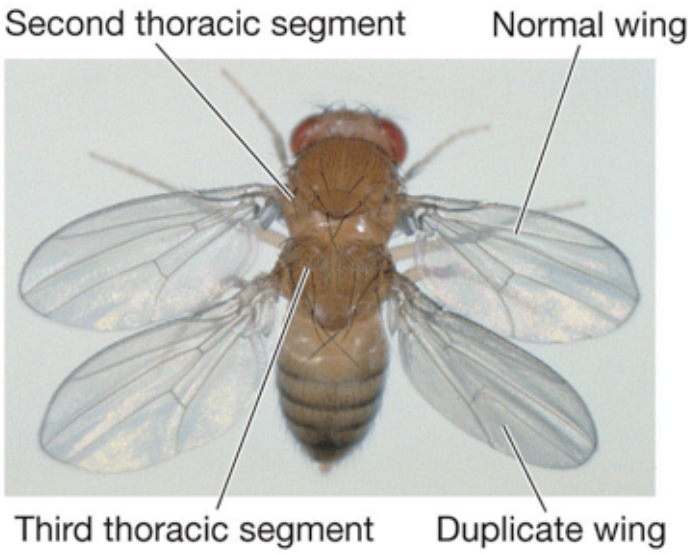


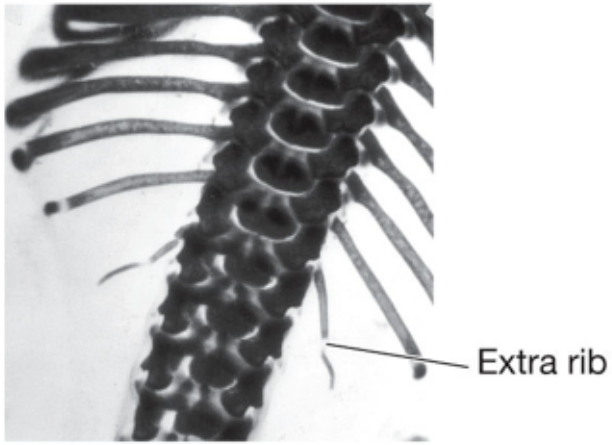
http://learn.genetics.utah.edu/content/variation/hoxgenes/
based on Wellik (2007)

Colinearity of genes and gene expression



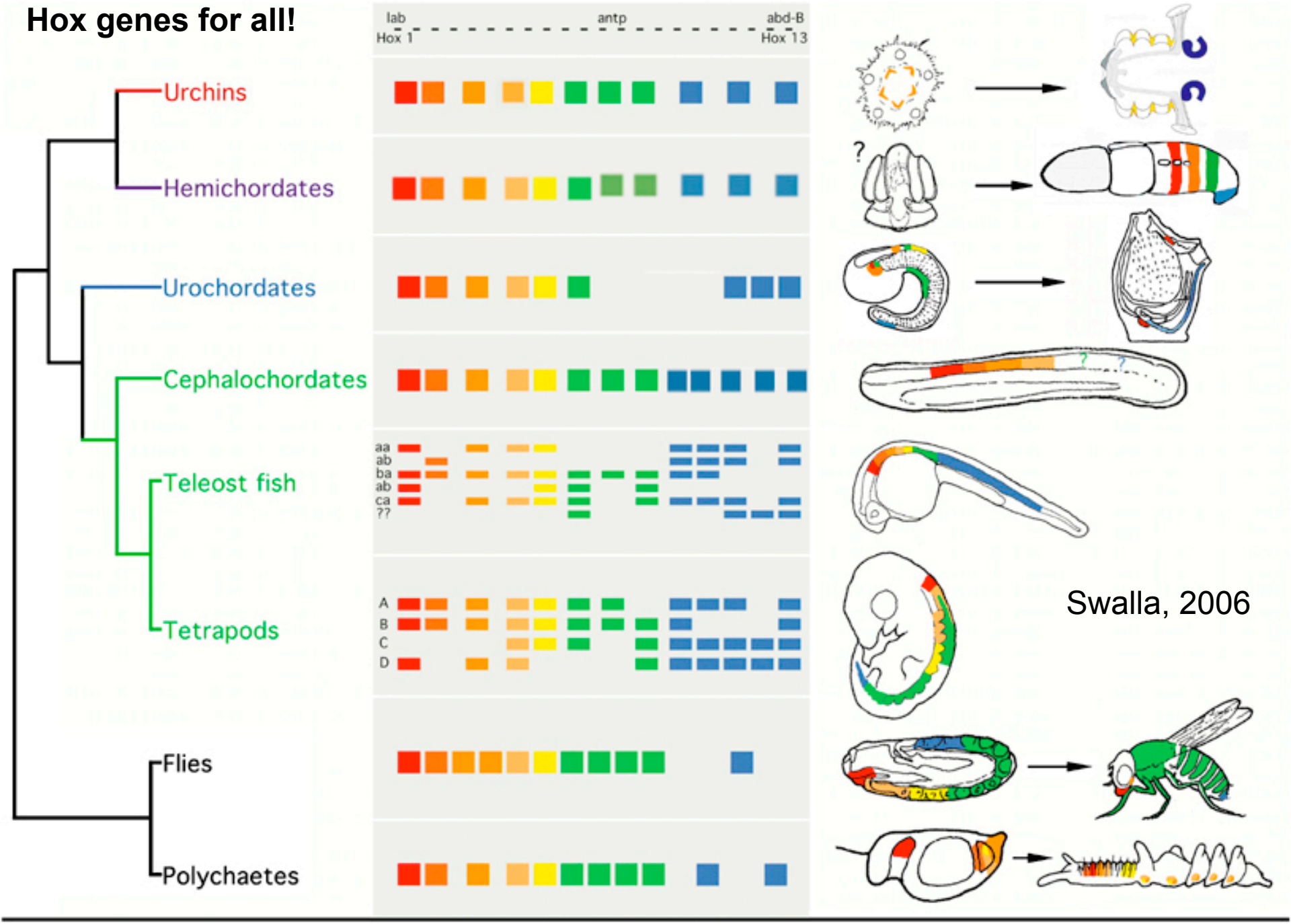
Hox genes cause homeotic phenotypes in all animals





lumbar vertebra is transformed into a thoracic vertebra

Hox genes for all!

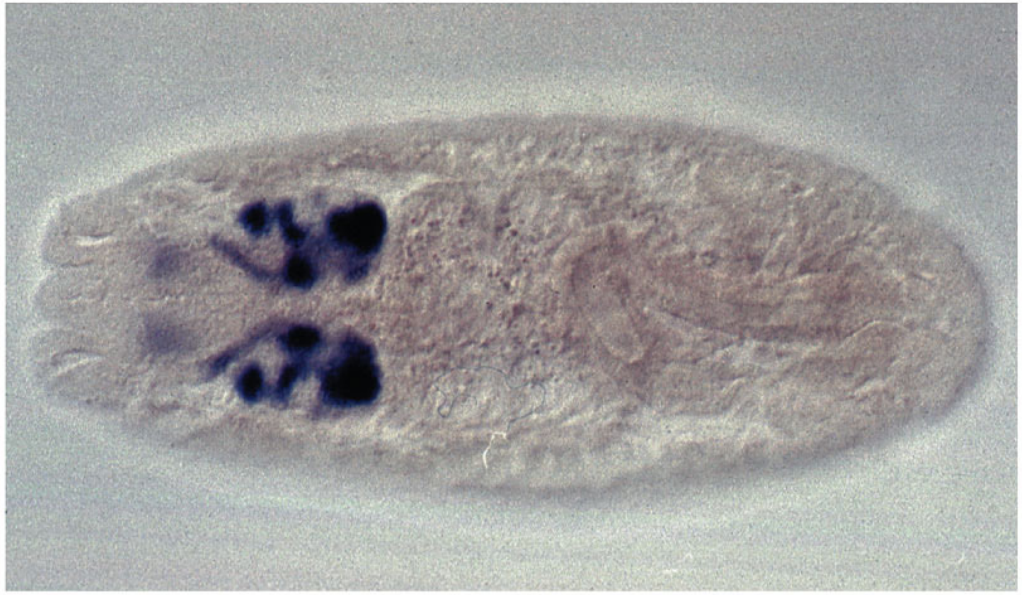


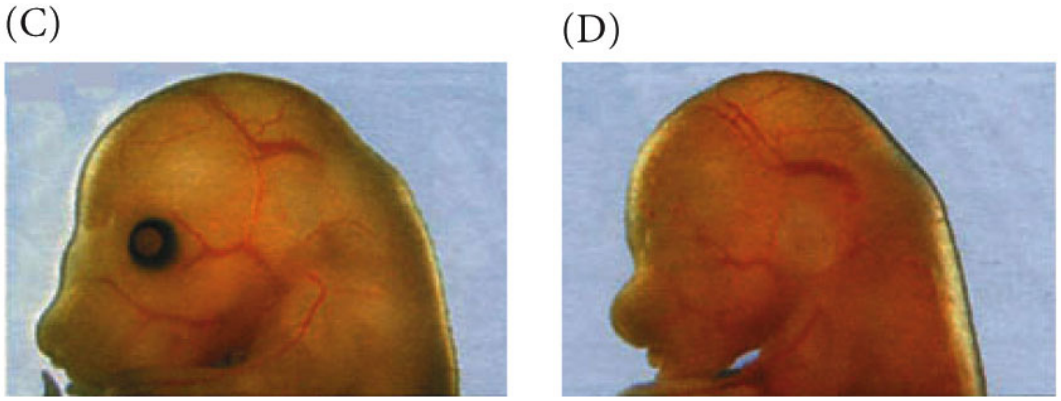
Swalla, 2006

the conserved "developmental toolkit"

- hox genes are just one example of how conserved developmental genes and processes are across evolutionary time

Expression of *eyeless* (*PAX6*) protein in *Drosophila*





Loss of eye in PAX6 mouse mutant

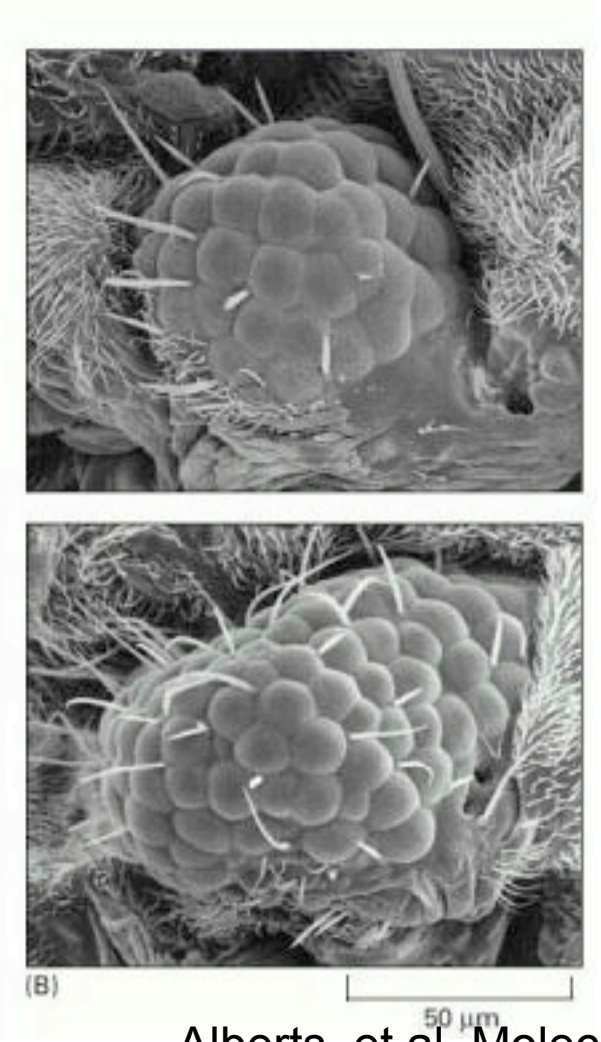
***EVOLUTION 2e*, Figure 21.9**

Pax6 is a highly conserved transcription factor involved in making eyes

supplying PAX6 from mouse and squid produces ectopic eye structures in *Drosophila*

mouse PAX-6 in fly

Futuyma,
***EVOLUTION 2e*, Figure 21.10**



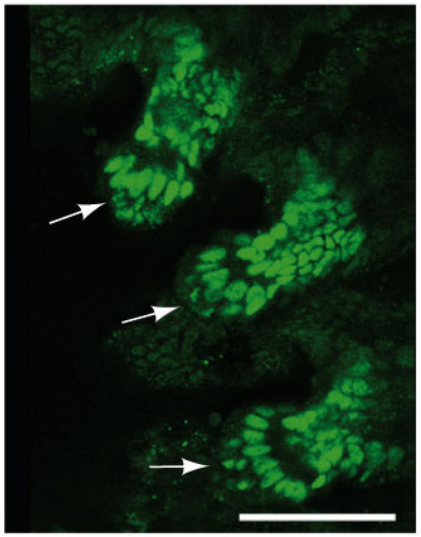
Drosophila Pax-6

squid Pax-6

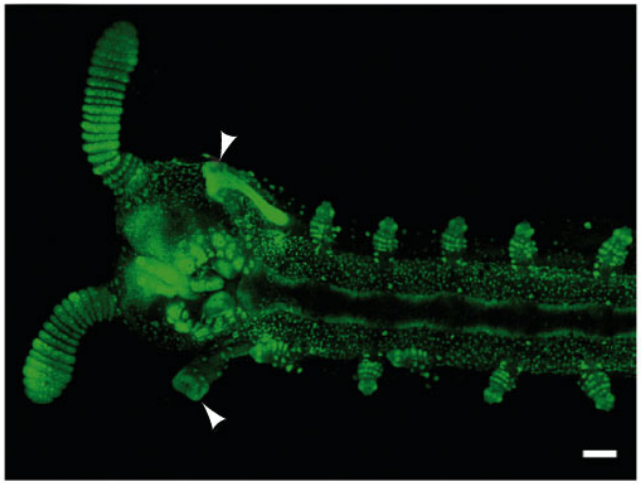
Alberts, et al, Molecular Biology of the Cell, 4th ed.

Expression of *Distal-less* family genes in the primordia of various animal appendages

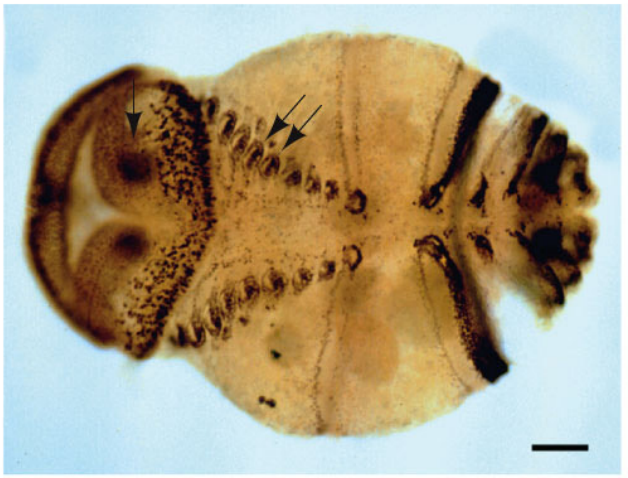
(A) butterfly larva



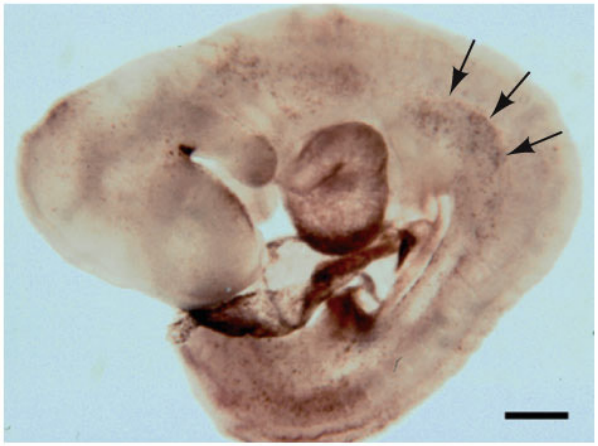
(B) velvet worm



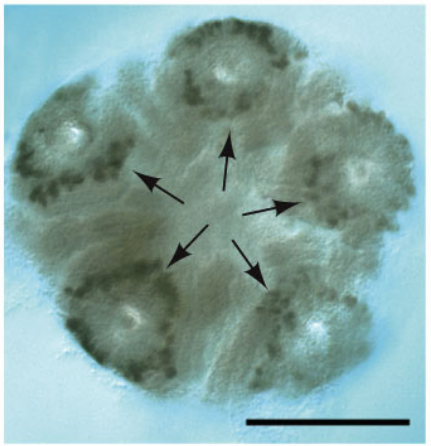
(C) marine polychaete embryo



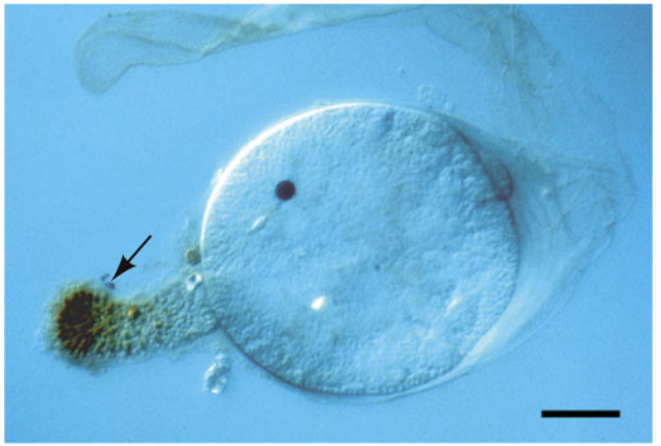
(D) mouse



(E) sea urchin larva



(F) larval sea squirt



arrows point to expression of *Distal-less* family genes

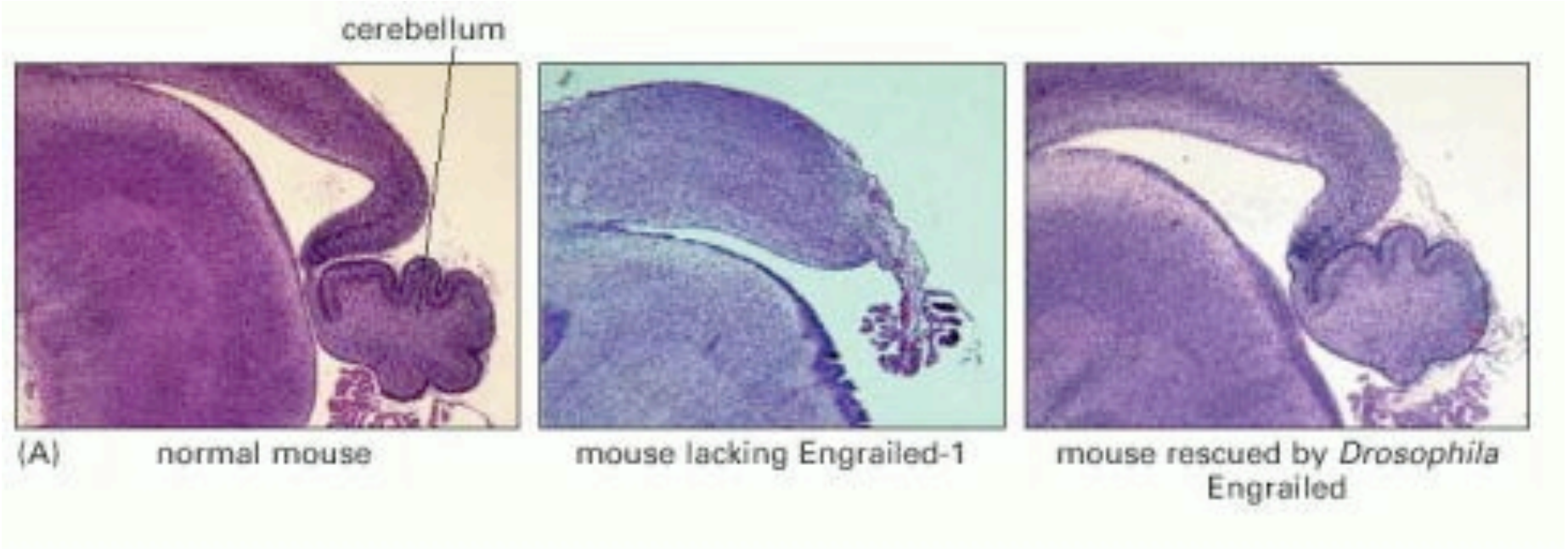
Futuyma,
***EVOLUTION 2e*, Figure 21.8**

the conserved "developmental toolkit"

- hox genes are just one example of how conserved developmental genes and processes are across evolutionary time

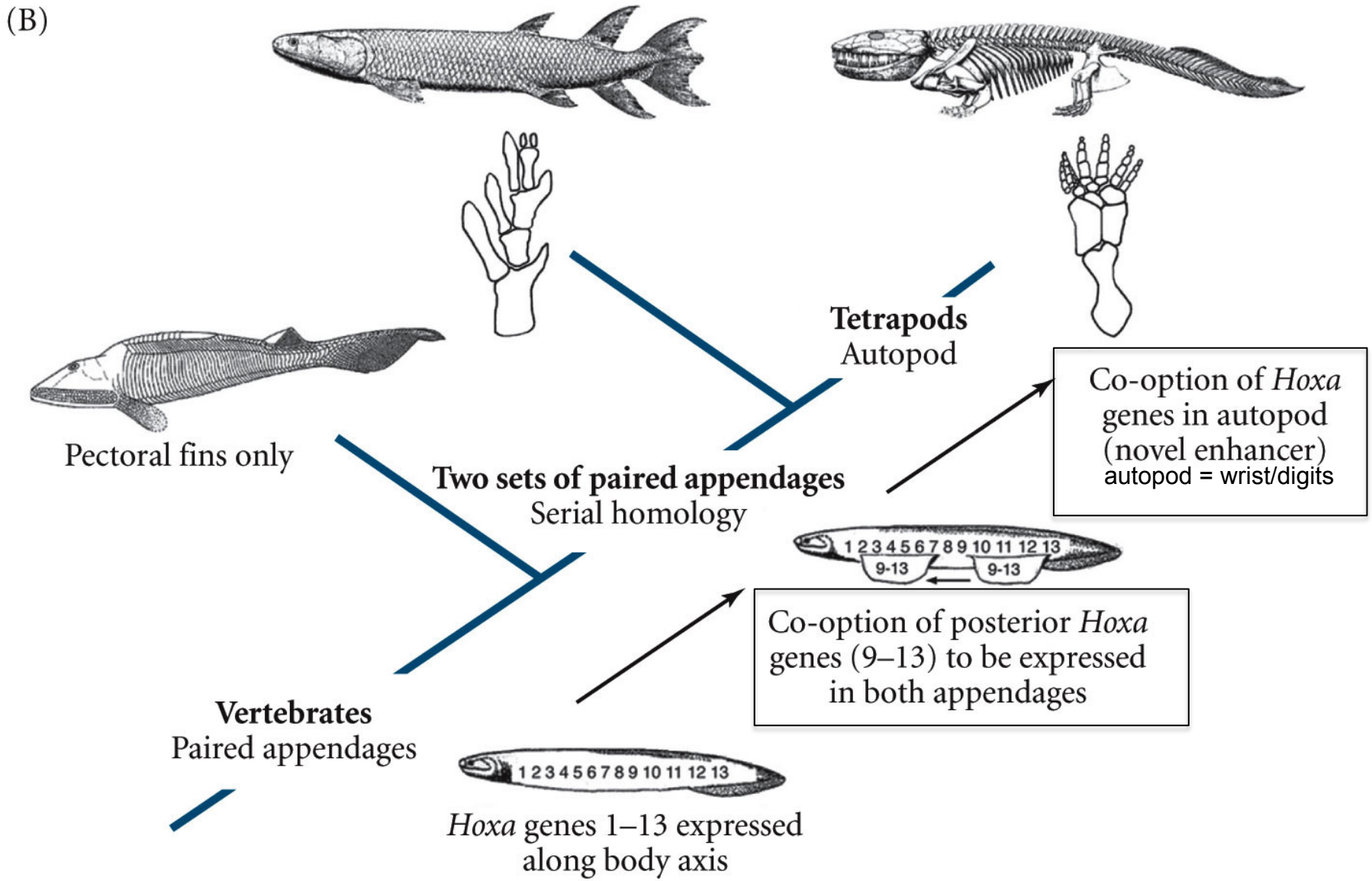
- these conserved genes and developmental processes can be altered to produce new structures

The *En* transcription factor is critical to producing the cerebellum in mouse

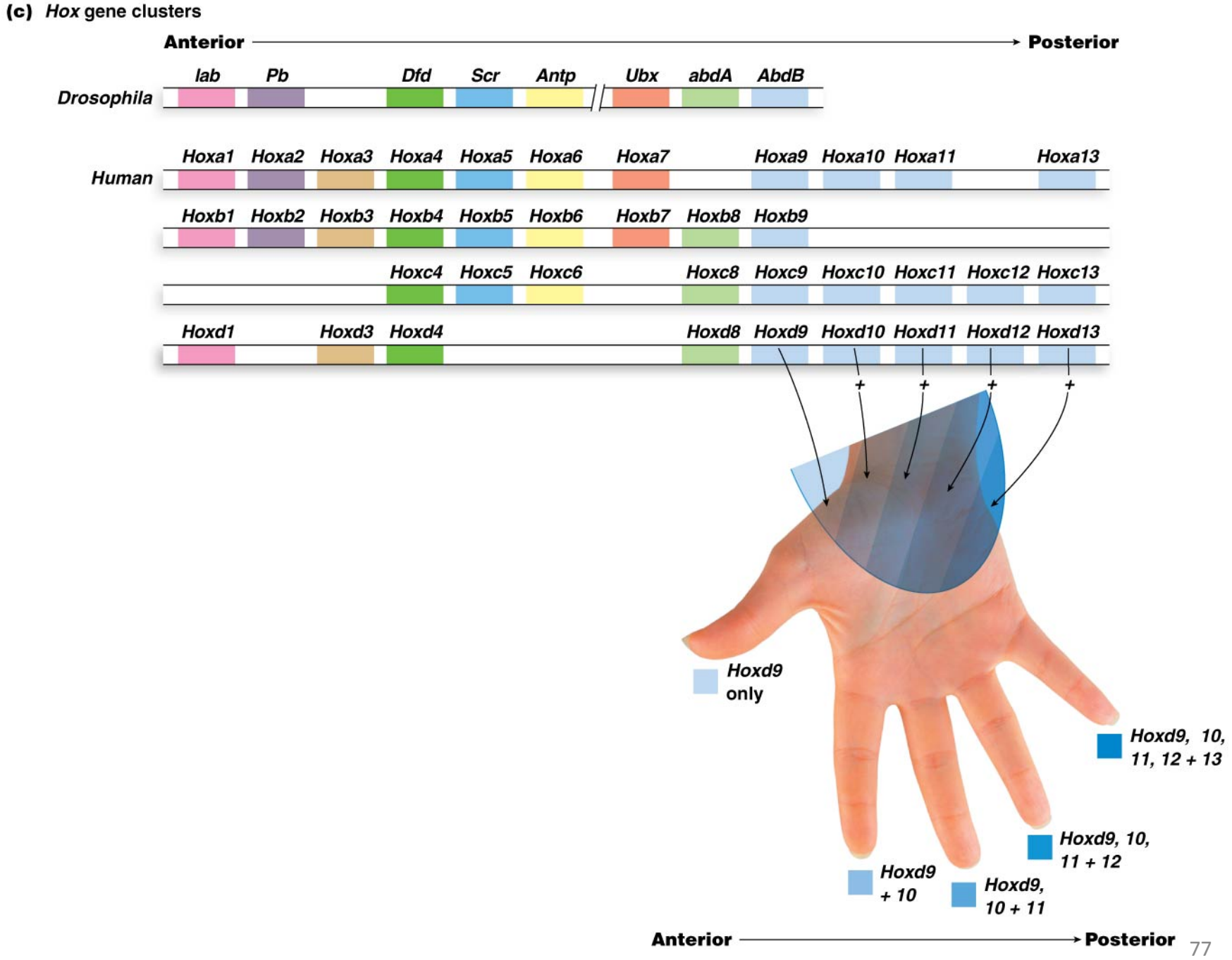


Alberts, et al, Molecular Biology of the Cell, 4th ed.

Co-option of developmental circuits in the evolution of novelties



EVOLUTION 2e, Figure 21.15 (Part 2)


(c) Hox gene clusters
Anterior
Posterior
lab
Pb
Dfd
Scr
Antp
Ubx
abdA
AbdB
Drosophila
Hoxa1
Hoxa2
Hoxa3
Hoxa4
Hoxa5
Hoxa6
Hoxa7
Hoxa9
Hoxa10
Hoxa11
Hoxa13
Human
Hoxb1
Hoxb2
Hoxb3
Hoxb4
Hoxb5
Hoxb6
Hoxb7
Hoxb8
Hoxb9
Hoxc4
Hoxc5
Hoxc6
Hoxc8
Hoxc9
Hoxc10
Hoxc11
Hoxc12
Hoxc13
Hoxd1
Hoxd3
Hoxd4
Hoxd8
Hoxd9
Hoxd10
Hoxd11
Hoxd12
Hoxd13
Hoxd9 only
Hoxd9, 10, 11, 12 + 13
Hoxd9, 10, 11 + 12
Hoxd9 + 10
Hoxd9, 10 + 11
Anterior
Posterior

Developmental genetics

- development and cell differentiation
- positional information
- *Drosophila* model
- Hox genes and the conserved developmental toolkit