Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

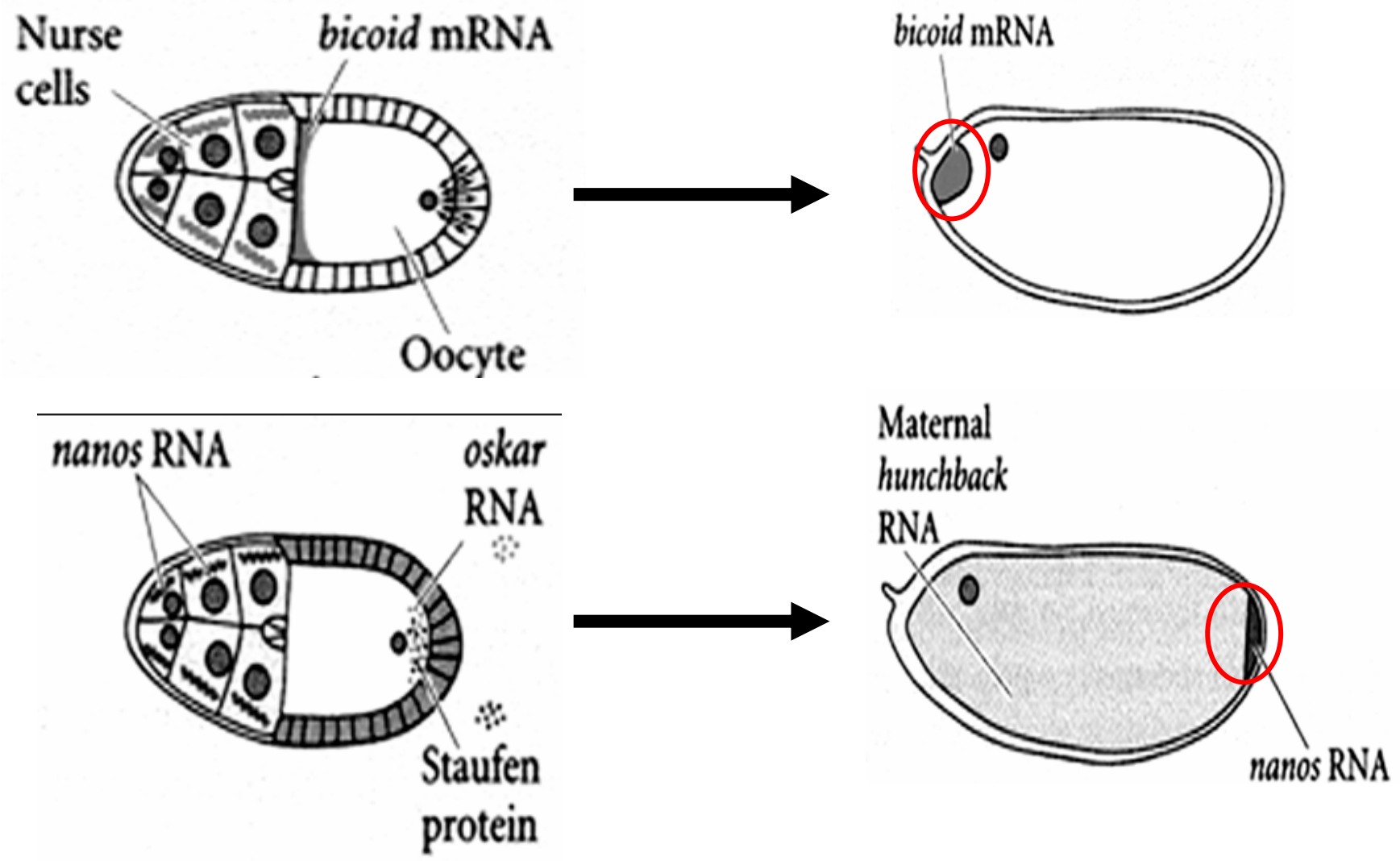
Lecture 26

Homeotic genes

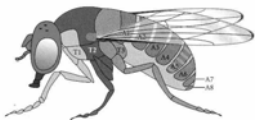
Normal fly

Ubx mutant

Antp mutant

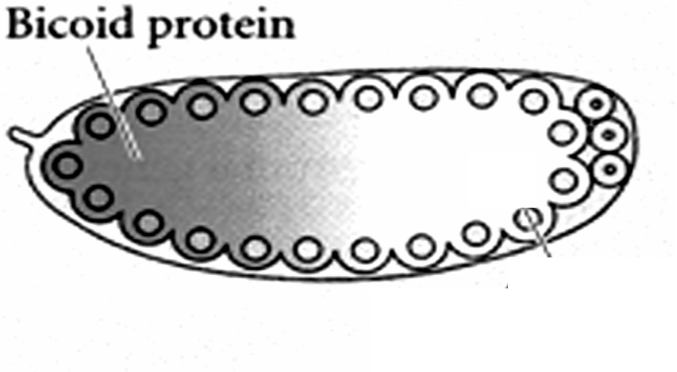


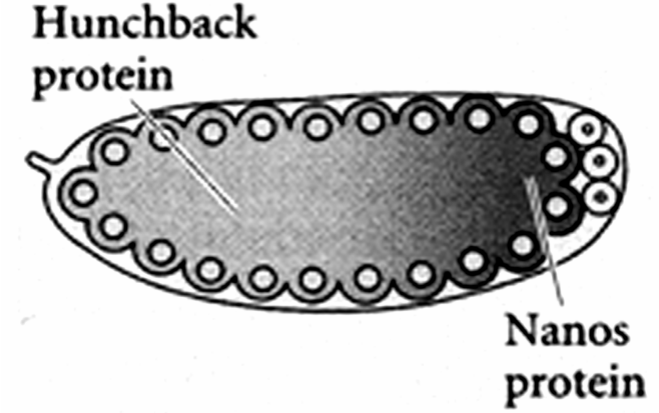
DIFFERENTIAL LOCALIZATION OF MATERNAL EFFECT GENE PRODUCTS IN THE OOCYTE CYTOPLASM BEFORE FERTILIZATION



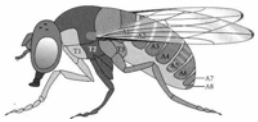
AFTER FERTILIZATION

- *bicoid* and *nanos* have opposite effects on a gene called *hunchback*
- *bicoid* is a transcription factor that activates *hunchback*
- *nanos* inhibits the translation *hunchback* mRNAs
- the combined effect of biocoid and nanos result in production of high levels of *hunchback* protein at the anterior of the embryo and low levels toward the posterior end





The combined concentration gradients of *bicoid*, *nanos* and *hunchback* sequentially activate or inhibit other morphogen genes in specific regions of the embryo



Signal transduction pathways

HEDGEHOG

ENGRAILED

NOTCH

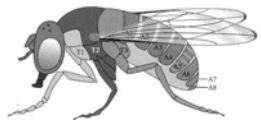
WNT

Fibroblast growth factor

TGF-beta superfamily

Platelet-derived growth factor

Ephrin



Summary of early expression of zygotic genes

A-P

bicoid protein gradient switches *hunchback* on at a high concentration

↓

hunchback activates and represses gap genes like *krüppel*, *knirps*, *giant*

↓

gap gene products and gap genes interact sharpen expression boundaries

↓

axis is divided into unique domains containing different combinations of transcription factors

D-V

gradient of intra-nuclear *dorsal* protein forms

↓

ventral activation of *twist*, *snail* and repression of *decapentaplegic*

↓

decapentaplegic expressed dorsally

↓

gradient of *decapentaplegic* activity patterns dorsal region

↓

dorso-ventral axis divided into prospective mesoderm, neurectoderm, epidermis amnioserosa

(Adapted from: www.chin-sang.ca)

Summary: pair-rule genes and segmentation

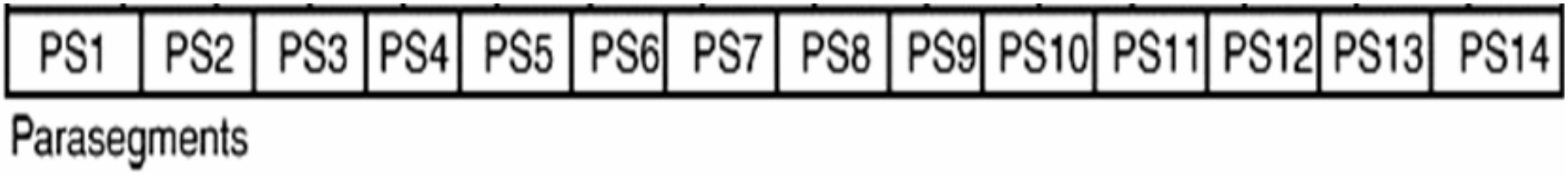
Production of local combinations of gap gene transcription factors

Activation of each pair-rule gene in seven transverse stripes along A-P

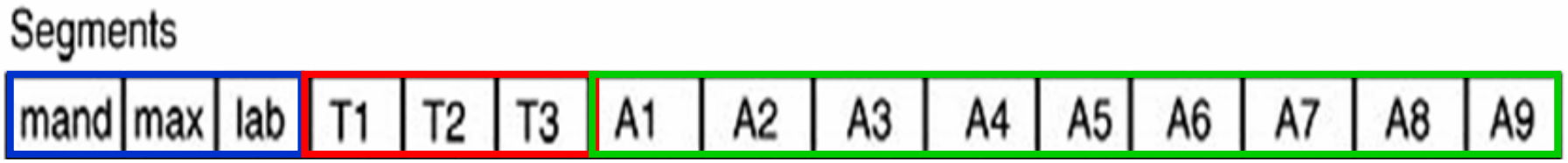
Pair-rule gene expression defines 14 parasegments, each pair-rule gene being expressed in alternated parasegments

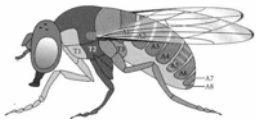
(Adapted from: www.chin-sang.ca)

By the action of products of the maternal, gap and pair-rule genes, *Drosophila* embryo is rapidly converted into 14 parasegments.



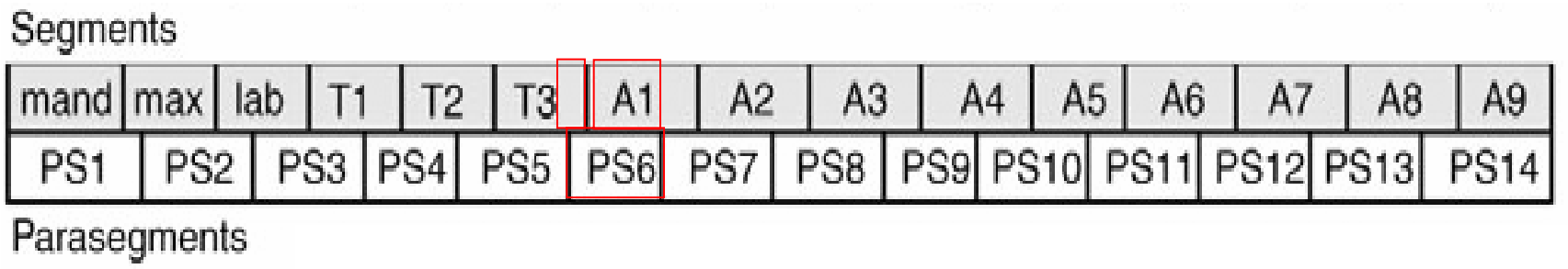
In adult fly, these 14 parasegments form the three head, the three thoracic and the eight abdominal segments.



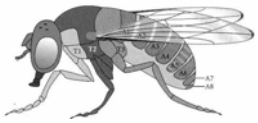


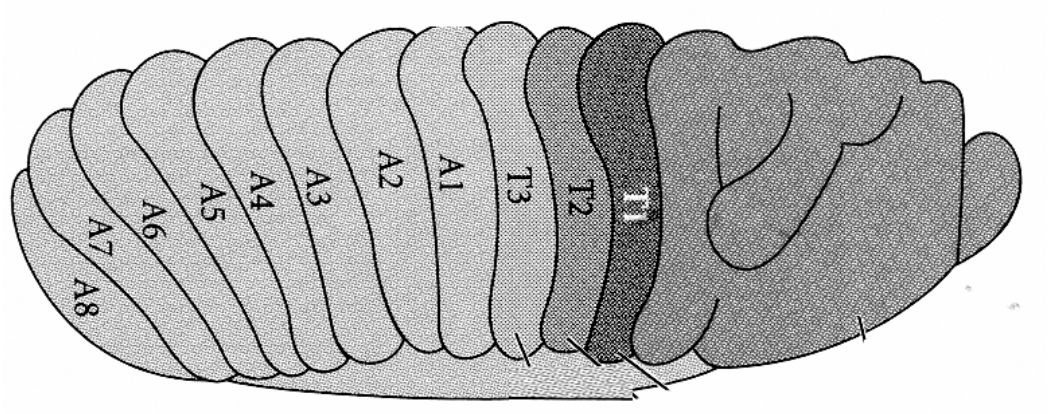
Although there are similar numbers of segments and parasegments, they are slightly shifted relative to one another. In the thorax and the abdomen, this shift is approximately half a segment.

For example, PS6 comprises the posterior of segment T3 and the anterior segment A1.

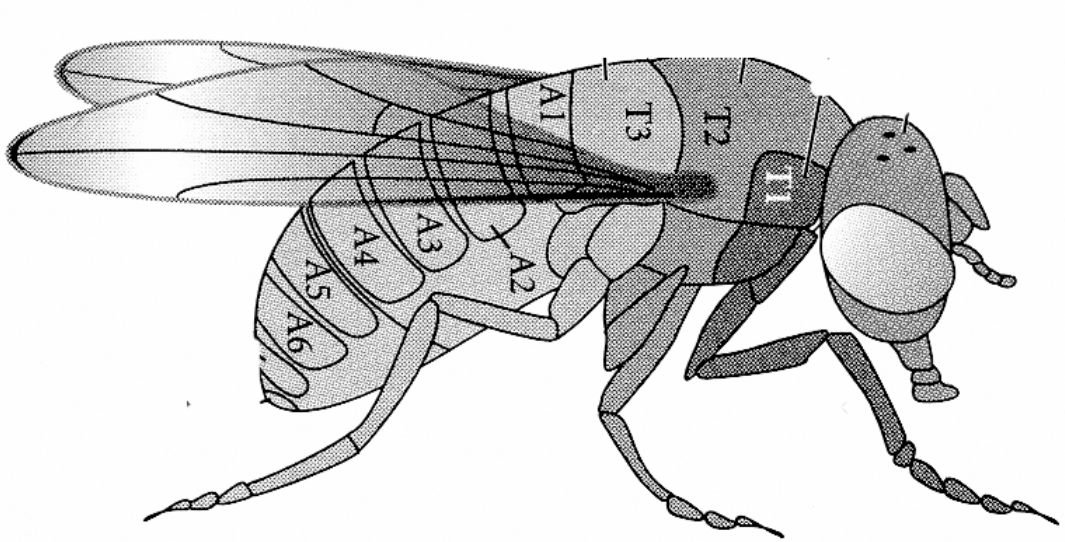


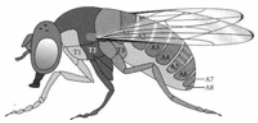
Thus, a parasegment comprises the posterior half of one segment and the anterior half of the next.





HOMEOTIC GENES





In Drosophila, eight homeobox genes regulate the identity of regions within the adult and embryo.

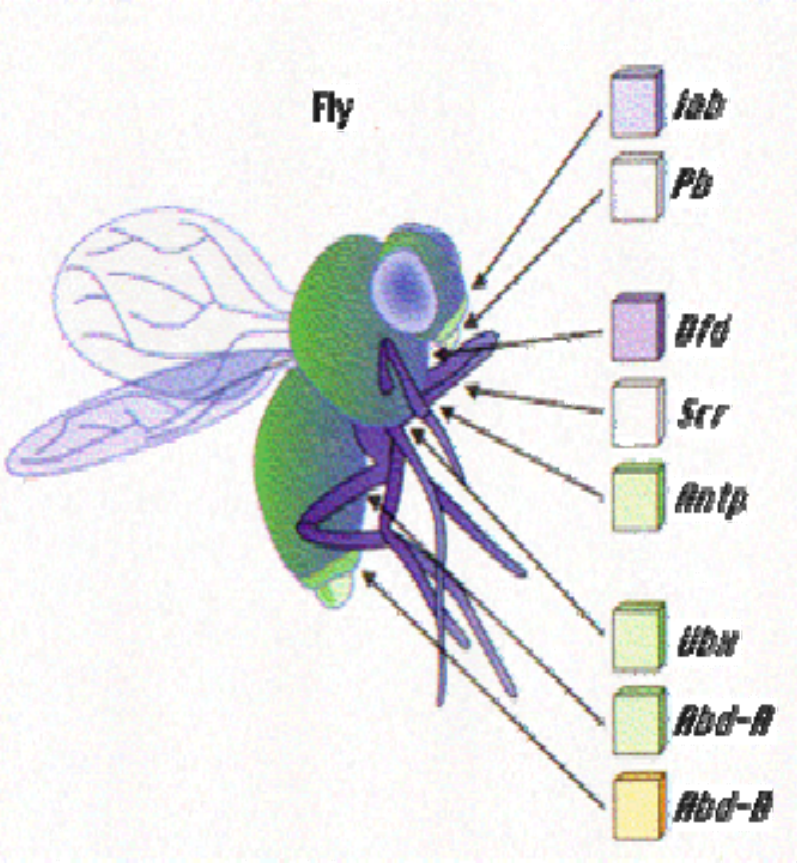
The Drosophila homeotic genes

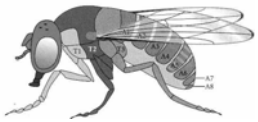
Antennapedia complex (Ant-C)

labial (lab)
Prodoscipedia (pb)
Deformed (Dfd)
Sex combs reduced (Scr)
Antennapedia (Antp)

Bithorax complex (Bx-C)

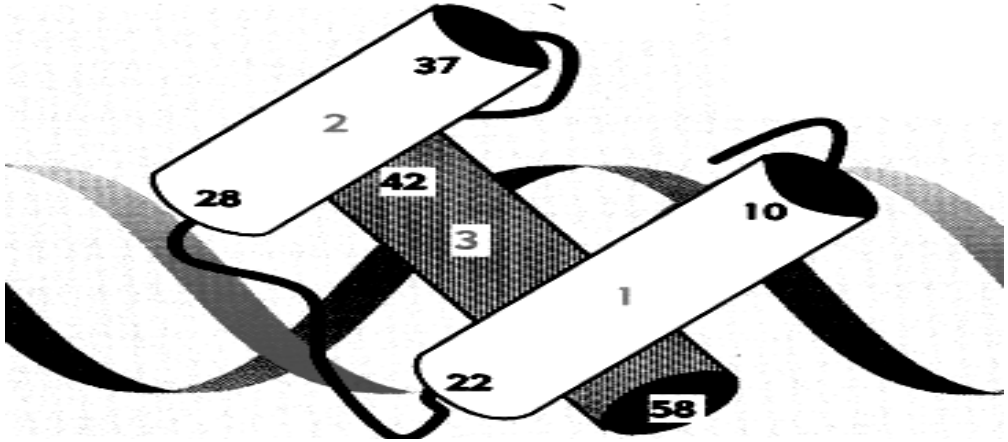
Ultrabithorax (Ubx)
Abdominal A (abdA)
Abdominal B (abdB)



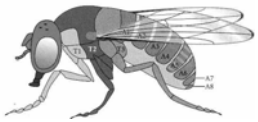


Homeotic gene products are transcription factors

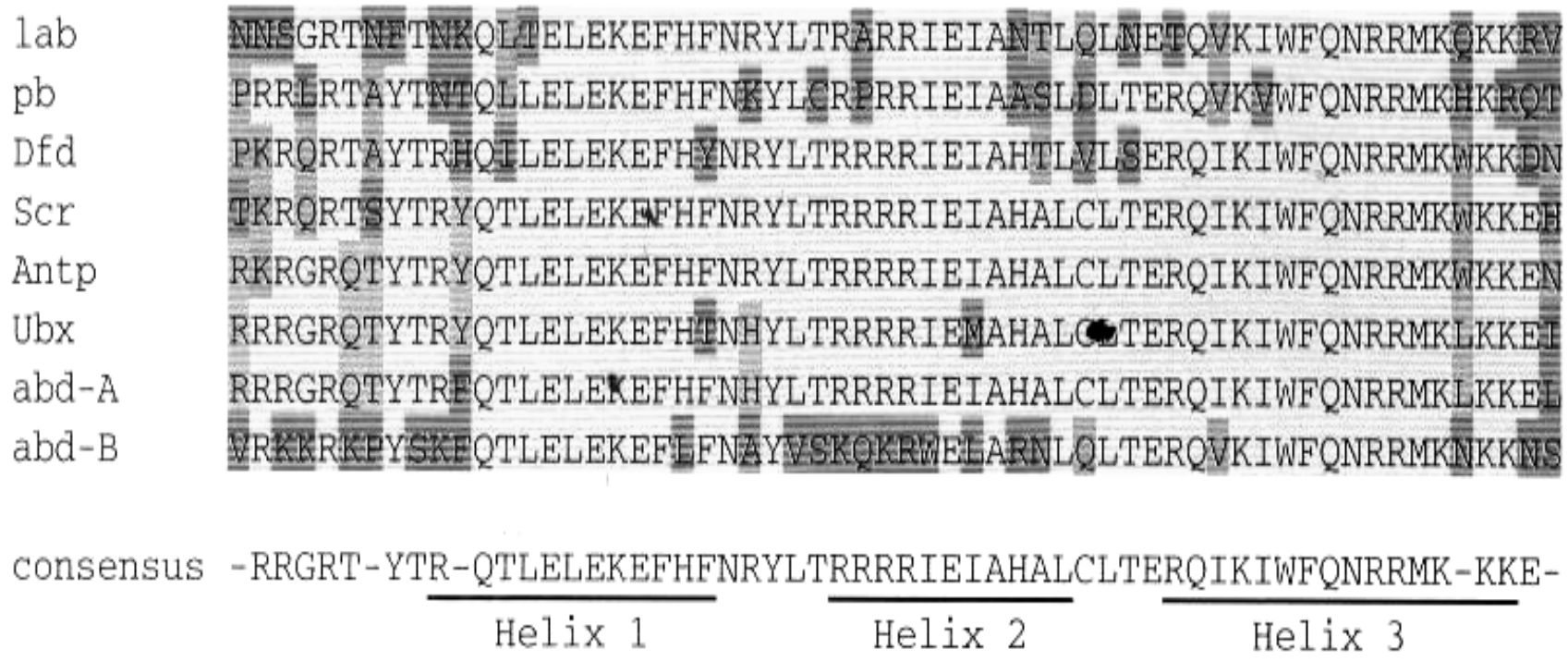
Homeotic genes contain a Homeobox sequence which codes for a 60 amino acid homeodomain that binds to specific DNA sequences at promoter and/or enhancerregions of target genes

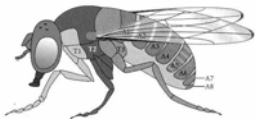


	1–10	11–20	21–30	31–40	41–50	51–60
Antp	RKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN					



Multiple alignment of the homeobox domains of Drosophila Hox proteins



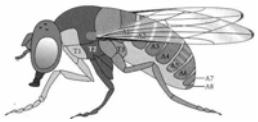


Homeotic genes act as “selectors”

i.e., they activate the construction of segment-specific traits like wings and legs on the thorax, antennae and eyes on the head etc.

For example, *antennapedia* (*Antp*) activates the construction of legs on the thoracic segments

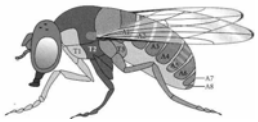
Antp is expressed in the thorax, but repressed in the head



Antennapedia complex (Ant-C)

The segments forming the head and the anterior thorax are determined by the Antennapedia complex (ANT-C).

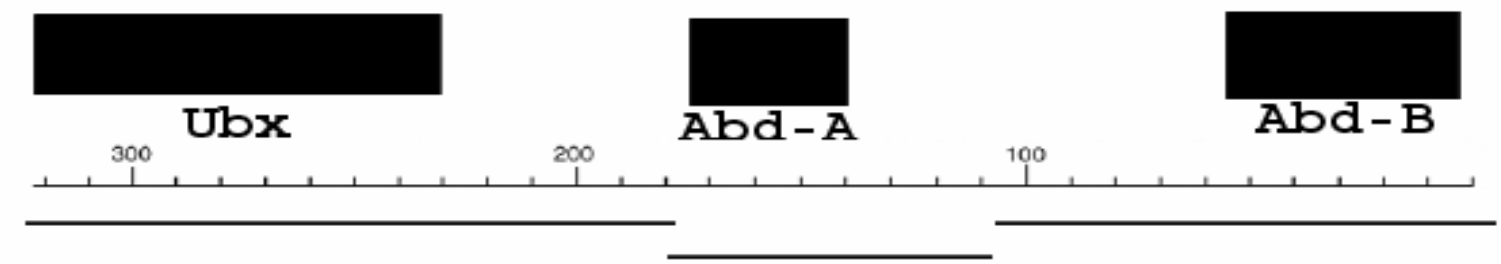
The ANT-C is named after the dominant gain-of-function phenotype of its founding member, *Antennapedia* (*Antp*), a mutant in which an extra pair of legs develop on the head instead of a normal pair of antennae



Bithorax complex (Bx-C)

The bithorax complex (BX-C) is responsible for determining the posterior thorax and each abdominal segment of the fly

The bithorax complex (BX-C) locus spans >300 kb and controls the identity of each of the segments that contributes to the posterior two-thirds of the fly. It consists olf three homeotic genes:
Ultrabithorax (*Ubx*), *abdominal A* (*abd-A*) and *Abdominal B (Abd-B*).



***Ultrabithorax* gene mutation transforms 3rd thoracic segment into another 2nd thoracic segment**

Proteins encoded by the gap and pair rule genes initiallly regulate the expression of these homoeotic genes. At later stages, proteins encoded by the Polycomb-Group (Pc-G) and trithorax-Group (trx-G) genes bind to these cis-regulatory elements and regulate the expression of these genes

Pc-G: -ve regulators;
trx-G: +ve regulators

The cis-acting elements to which the gap and pair-rule proteins bind are known as initiators, and the elements to which the Pc-G and trx-G proteins bind are termed maintenance elements (ME) or Pc-G response elements (PREs) or trx-G response elements (TREs)].

Both the Pc-G and trx-G products maintain the active or inactive state of each parasegment-specific cis-regulatory region by modifying the chromatin structure of each region.

The Polycomb-Group (Pc-G) genes (~40 genes) keep the homeotic genes of the BX-C repressed in those segments where they have not been activated during early embryogenesis.

The Pc-G gene products form large complexes that package chromatin into a compact, transcriptionally inactive conformation

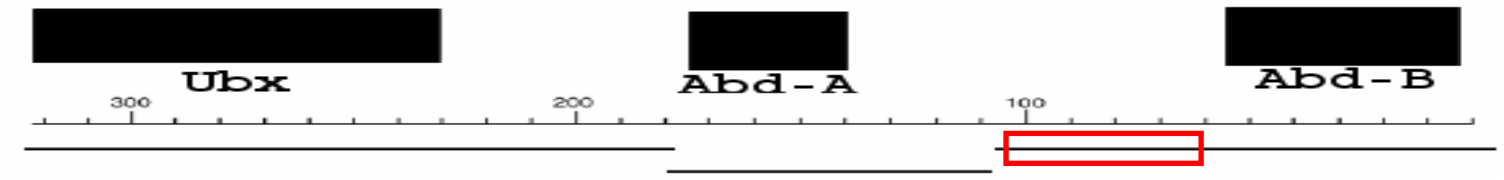
There are two Pc-G protein repressor complexes: PRC1 and PRC2

The PRC2 complex has a histone H3 Lys 27 (H3-K27) methyltransferase activity.

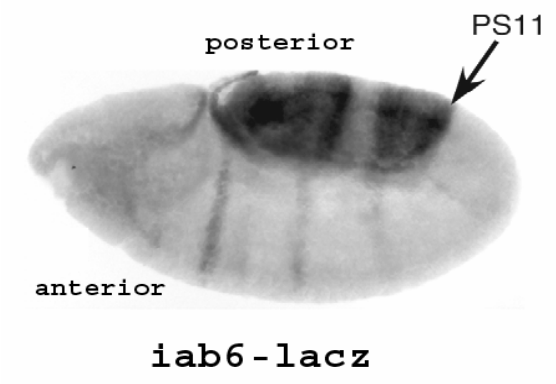
H3-K27 is an epigenetic chromatin modification that is associated with transcriptionally inactive chromatin

The trithorax-Group (trx-G) genes counter to the Pc-G genes by maintaining the homeotic genes and their large cis-regulatory regions in a transcriptionally permissive state.

All the trx-G gene products have chromatin modification activities.



BX-C gene regulation was studied cloning the various regulatory regions upstream of a *lacZ* reporter gene, making transgenic flies carrying these reporter constructs and studying their resulting patterns of expression



Iab6 region is responsible for Abd-B expression in PS11

Based on such studies, specific DNA fragments that are required for initiating the segment-specific expression of BX-C genes, maintaining the restricted pattern of their expression, and for producing segment-independent, cell-type specific expression were identified.

Binding sites for Hox proteins are frequently juxtaposed to binding sites for other transcription factors, like effectors of signaling pathways, or proteins that determine tissue-specific expression

For ex., binding of the SOX/OCT heterodimer close to the sequences bound by HoxB1 is required for the full transcriptional activity dictated by this sequence

The Hox genes can alter the expression of ligands, receptors, intermediate elements or effectors of signaling pathways.

For instance, in *Drosophila,* Ubx regulates the activity of the Notch, Hedgehog, Dpp, Wingless (Wg) and Epidermal growth factor receptor (EGFR) signaling pathways in the haltere disc (with respect to the wing one) to make a haltere instead of a wing

Similarly, proboscipedia determines pattern differences between the proboscis and the leg by modifying the Hedgehog pathway

Another example is the formation of denticles in the embryonic ventral cuticle of the *Drosophila* embryo.

The abdominal segments bear more rows of denticles, which are also of bigger size, than the thoracic ones.

This is due to Ubx and abd-A activating in the abdomen a ligand of the Notch pathway, Serrate, which, in turn, triggers EGFR signalling to form denticles.

In the thorax, the Hox genes do not activate Ser expression, there is no EGFR signaling and the denticle bands are thinner and with more slender denticles

Complex cooperative interactions between signaling pathways and Hox genes play a key role in inducing the expression of target genes.

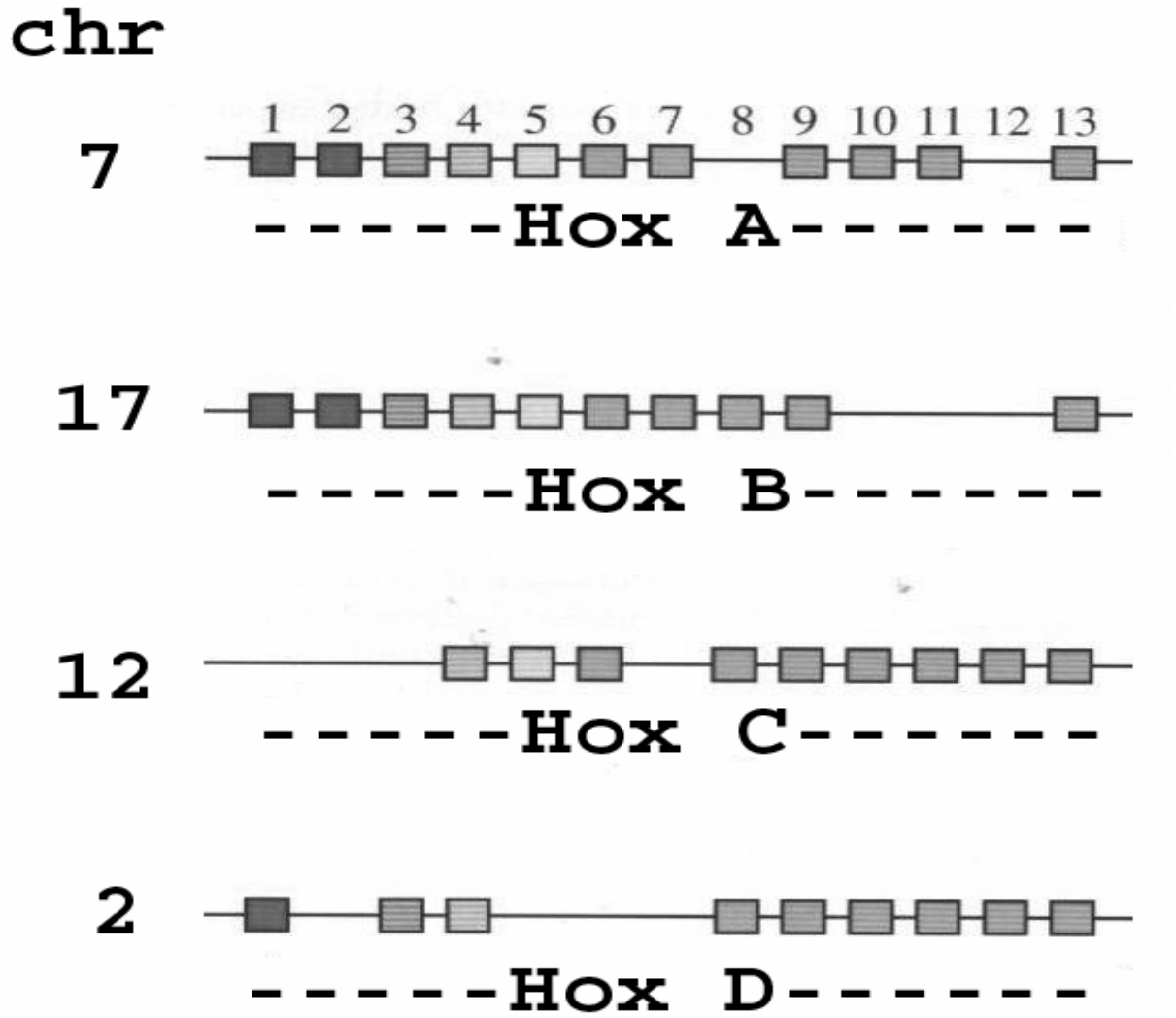
For ex., protein-protein interactions between, HoxD proteins and Gli-3, a member of the Sonic hedgehog signaling pathway specify digits in vertebrate limb.

The relationship between hox genes and signalling molecules can be very complicated.

For ex., Hox genes can be both upstream as well as downstream of the Sonic Hedgehog pathway in the formation of digits in the mouse

Vertebrates have 4 Hox clusters

Human chromosomes

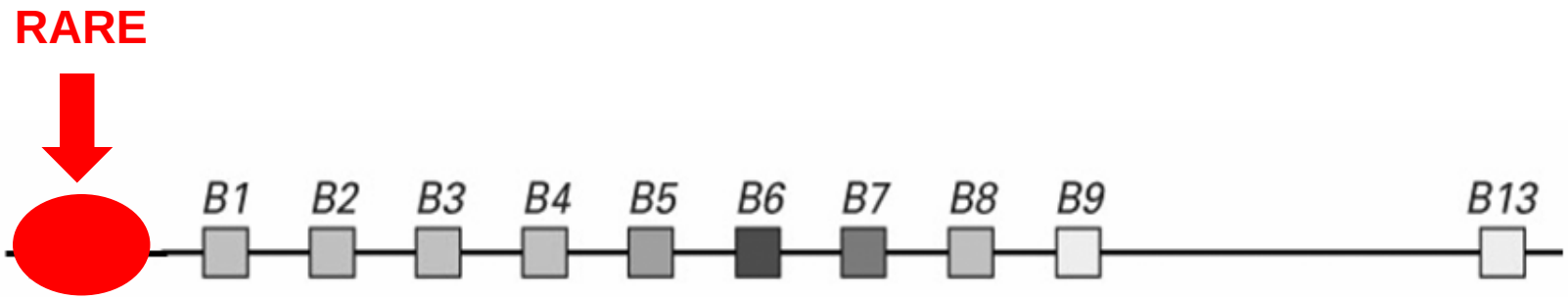


Vertebrates Hox genes display colinearity:

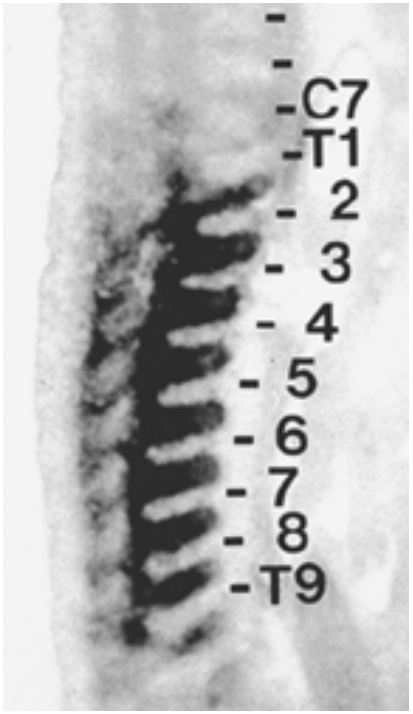
a) Temporal colinearity: genes on one end of the complex are expressed first, those on the other (posterior) end are turned on last.

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

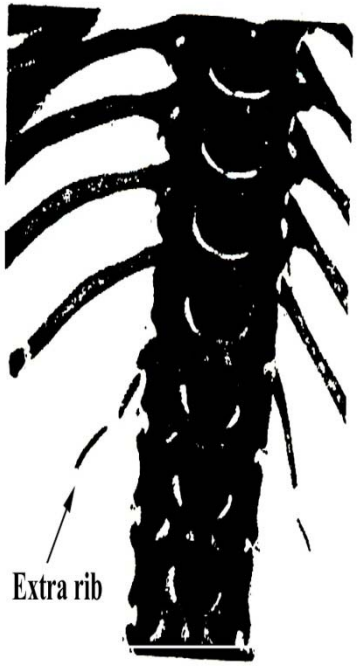
c) Anterior Hox genes are activated sequentially by retinoic acid.



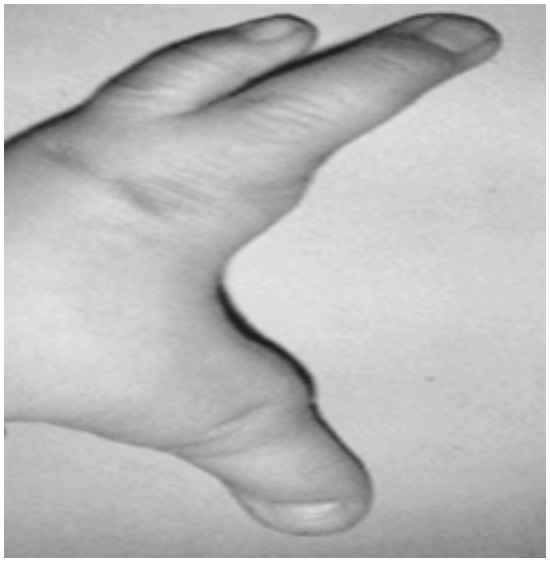
Hox-C6 protein is expressed in eight thoracic segments of the mouse embryo.

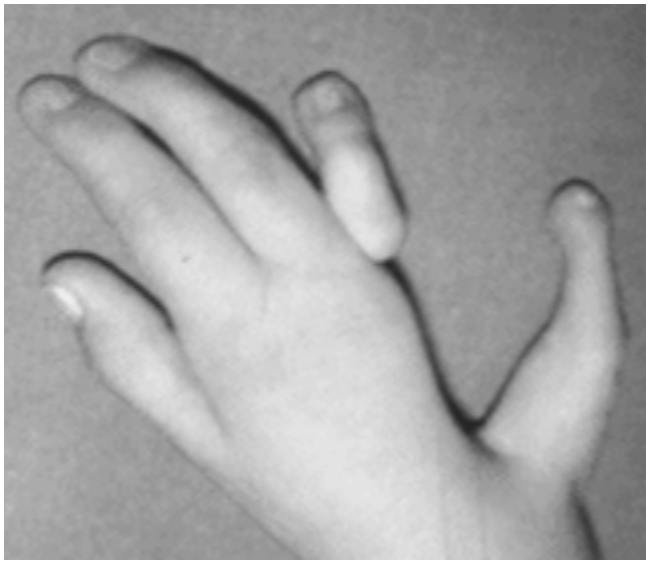


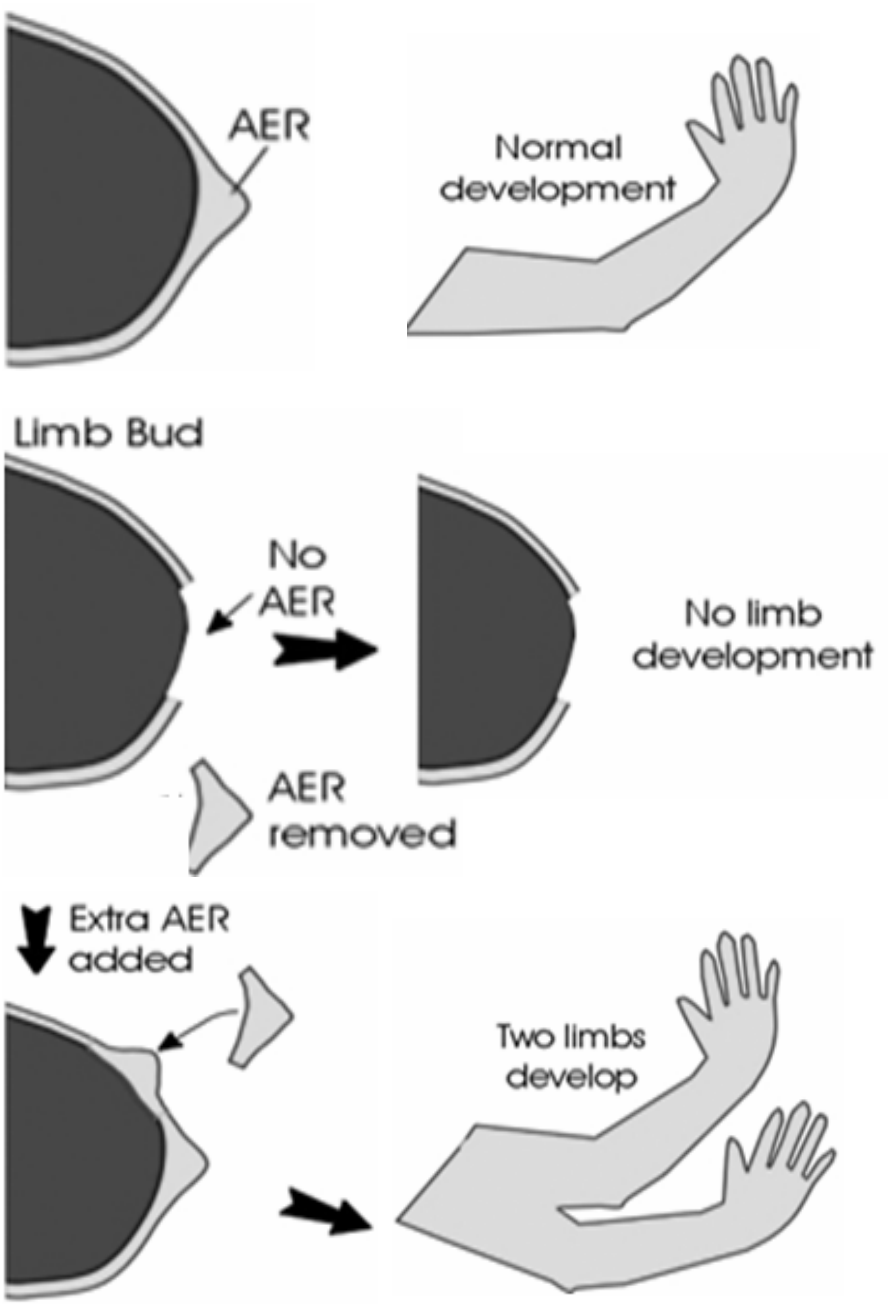
Mutations in HoxC-8 can lead to the development of an extra rib in the lumbar region.



Mutations in specific Hox genes can lead to abnormal limb development







The distal tip of the limb bud is covered with a transient structure known as the **apical ectodermal ridge (AER)**. If the AER is removed, the mesoderm stops dividing.

If a supernumerary AER is grafted adjacent to a developing limb, a supernumerary limb results.

If limb bud mesoderm is removed from an early limb bud, the AER regresses and the mesoderm ceases proliferation.

<u>Limb development involves reciprocal interactions between the AER and underlying mesoderm.</u>

The limb has three axes: Proximo-distal; Anterior-posterior and Dorsal-ventral.

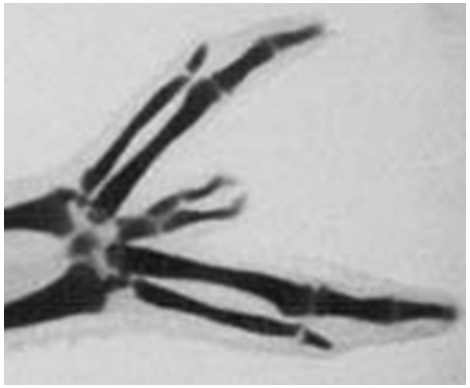
Anterior-Posterior Axis

This axis is determined by the **zone of polarizing activity (ZPA)**, which is located at the junction between the limb bud and the body wall.

Grafts of the ZPA to the anterior margin of a host limb bud causes duplication of digits in mirror-image symmetry.

Retinoic acid released from the ZPA plays a crucial role in limb development

A bead soaked in Retinoic Acid when implanted into the anterior margin of the early wing limb-bud results in mirror-image duplication of the digits.



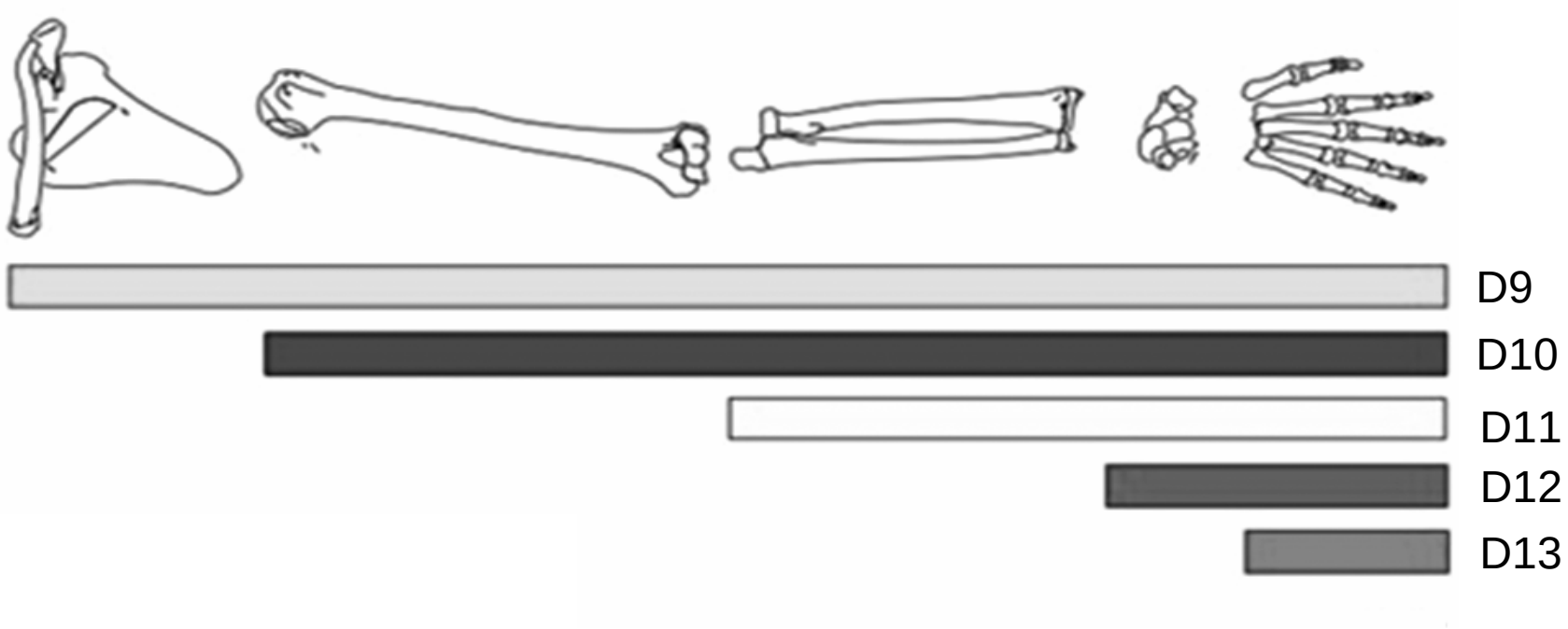
IV
III
II

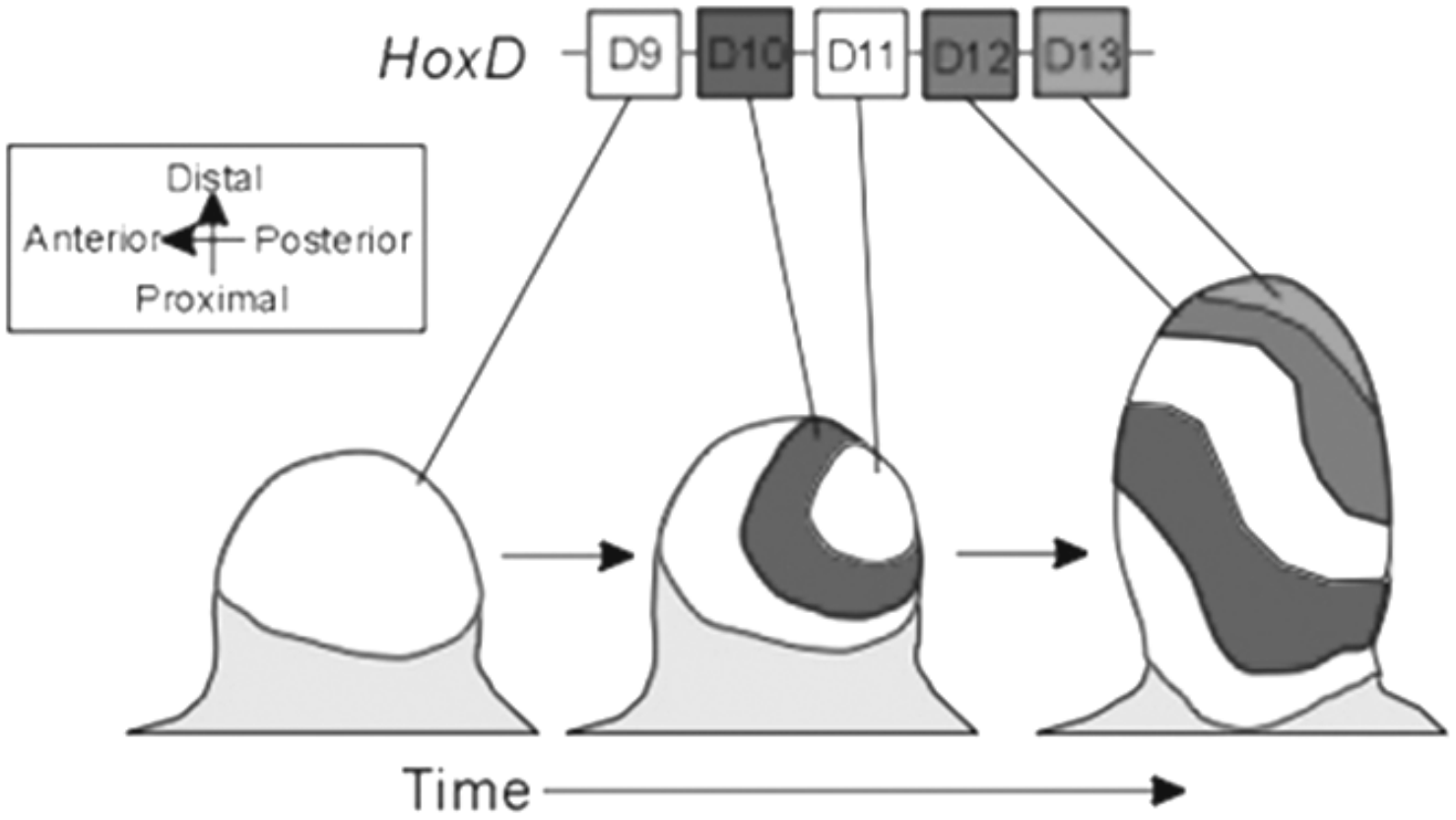
___

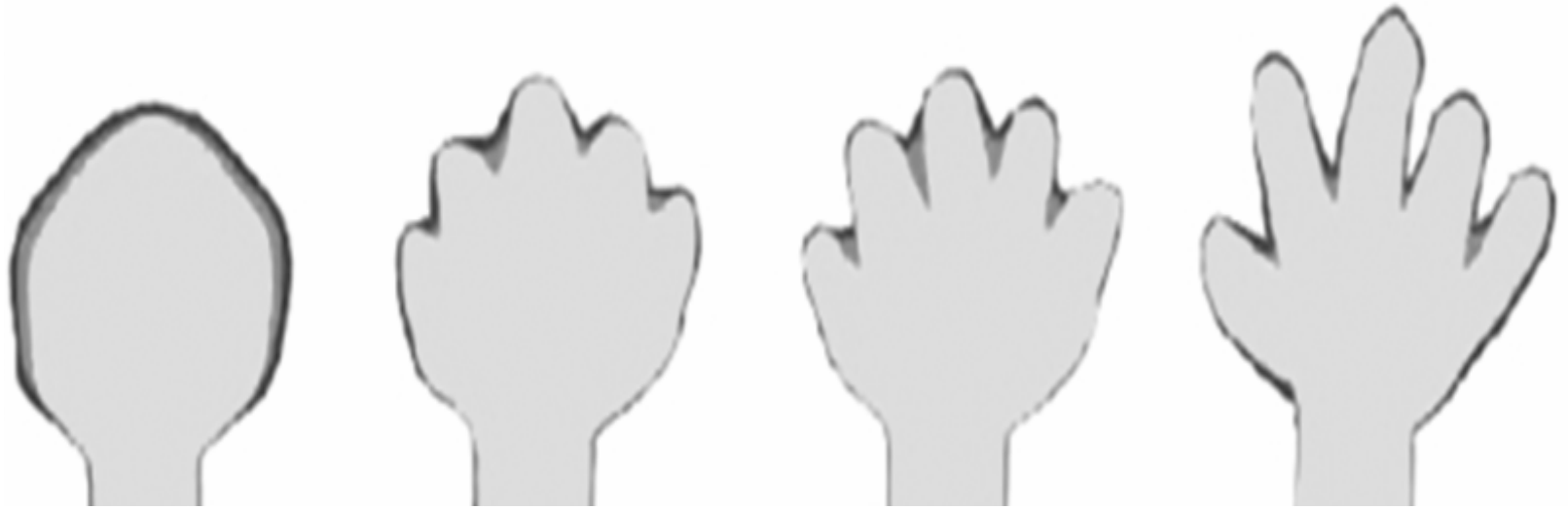
II
III
IV

Digit IV represents a posterior limb structure.
The ectopic release of Retinoic Acid from the bead leads to ectopic expression of **Sonic Hedgehog (SHH)**, forming a secondary ZPA.

The HoxD gene complex is expressed in a specific pattern in the developing mouse forelimb..




HoxD
D9
D10
D11
D12
D13
Distal
Anterior
Posterior
Proximal
Time



Differential gene expression during vertebrate eye development

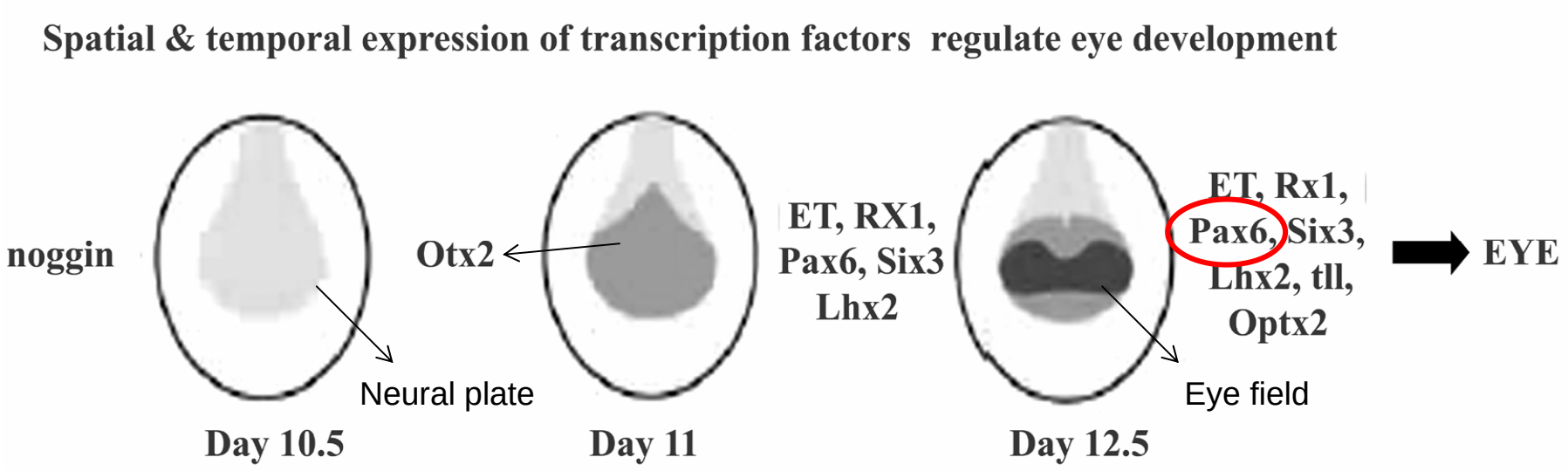
The development of eye is specified at the neural plate stage when a group of eye field transcription factors, EFTFs, are expressed in the anterior neural plate.

The EFTFs include *ET, Rx1, Pax6, Six3, Lhx2, tll* and *Optx2.*

Mutations of *PAX6, SIX3* and *OPTX2* in human result in abnormal eye development.

Mutation of *Pax6, Rx* , *Lhx2, Tll, Six3* and *Six6* in mouse, results in animals with abnormal or no eyes. Similar phenotypes have been observed when homologues of *Six3, Pax6, tll, Rx1* and *Optx2* genes were functionally inactivated in other vertebrates.

These EFTFs are not only necessary for eye formation, but also sufficient since overexpression of *Pax6, Six3, Rx* and *Optx2* homologues can induce eye tissues in the nervous system of vertebrates.



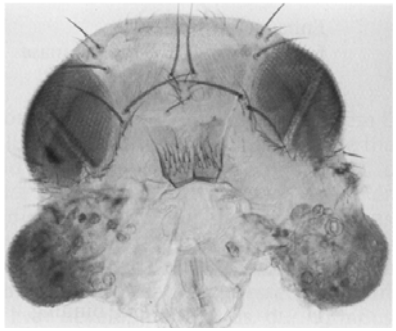
Adapted from:

Specification of the vertebrate eye by a network of eye field transcription factors
Michael E. Zube ME et al., (2003) Development 130, 5155-5167

Pax-6 and eye development

Paired box (Pax) genes are a family of tissue specific transcription factors containing a paired domain and a homeodomain.

Pax group 1 (Pax 1 and 9),
Pax group 2 (Pax 2, 5 and 8),
Pax group 3 (Pax 3 and 7) and
Pax group 4 (Pax 4 and 6).



Mutation or deletion of one of the pax-6 alleles (heterozygote) results in complete absence of the iris of the eye (The iris is like the aperture of a camera. It's loss results in inability to control light entering the eye).

When both alleles of Pax-6 are altered (homozygous), the result is a near complete failure of entire eye formation and often the fetuses die prior to birth with severe brain damage.

Mutations in genes encoding Transcription factors result in abnormal phenotypes

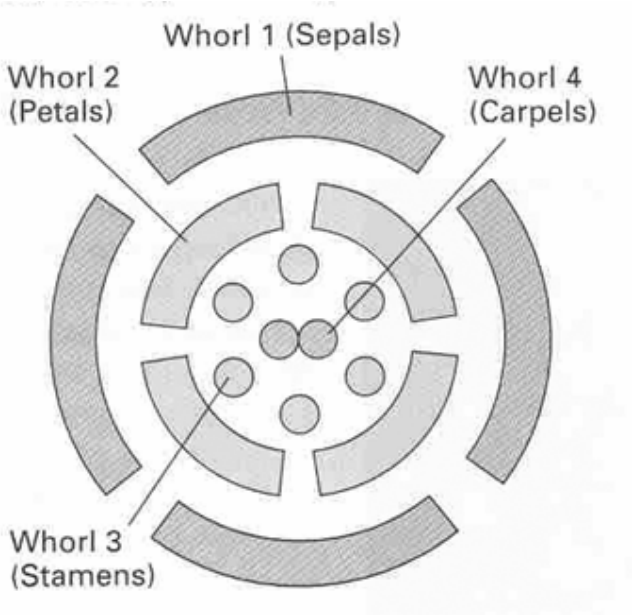
• Androgen receptor	Androgen insensitivity syndrome
• *AZF1*	Azoospermia
• *CBFA1*	Cleidocranial dysplasia
• *CSX*	Heart defects
• *EMX2*	Schizencephaly
• Estrogen receptor	Growth reg. problems, …
• *Forkhead-like 15*	Thyroid agenesis, cleft palate
• *Gl13*	Grieg syndrome
• *HOXA-13*	Hand-foot-genital syndrome
• *HOXD-13*	Polysyndactyly
• *LMXIB*	Nail-patella syndrome
• *MITF*	Waardenburg syndrome type 2
• *Pax2*	Renal-coloboma syndrome

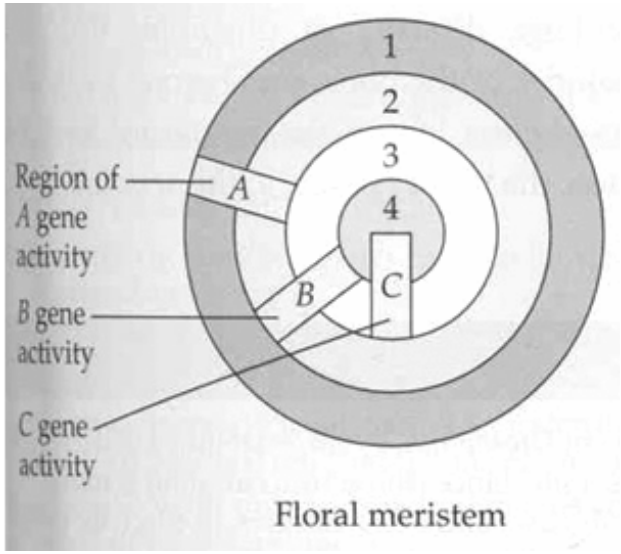
Mutations in genes encoding transcription factors result in abnormal phenotypes

- *PAX3* Waardenburg syndrome type 1
- *PAX6* Aniridia
- *PTX2* Reiger syndrome
- *PITX3* Congenital cataracts
- *POU3F4* Deafness and dystonia
- *SOX9* Campomelic dysplasia, male sex reversal

Plant homeotic genes encode MADS box containing transcription factors and change floral organ identity when they are mutated.

Three classes of *floral organ-identity genes* in *Arabidopsis:* A B and C.
They encode transcription factors of MADS family





Active genes	Organs formed
A	Sepals
A+*B*	Petals
B+*C*	Stamens
C	Carpels

Regulatory mechanisms for floral homeotic gene expression.
Liu Z, Mara C.
Semin Cell Dev Biol. 2010 Feb;21(1):80-6. Epub 2009 Nov 13. Review.

Beyond the ABCs: ternary complex formation in the control of floral organ identity.
Gutierrez-Cortines ME, Davies B.
Trends Plant Sci. 2000 Nov;5(11):471-6. Review.

Lewis, E. B. (1978).
A gene complex controlling segmentation in *Drosophila*.
Nature **276**, 565-570.

Robert K. Maeda and François Karch (2006)
The ABC of the BX-C: the bithorax complex explained
Development 133, 1413-1422

Veraksa A, *et al*. 2000. Minireview: Developmental Patterning Genes and Their Conserved Functions: From Model Organism to Humans. Molecular Genetics and Metabolism 69:85-100

Ingham and McMahon 2001. Hedgehog signaling in animal development: paradigms and principles. Genes & Development 15: 3059-3087.

Kim, Kim, and Hui. 2001. The VACTERL Association: lessons from the Sonic Hedgehog pathway. Clin. Genet. 59:306-315.

Transcriptional regulation of early oogenesis: in search of masters.
Pangas SA, Rajkovic A.
Hum Reprod Update. 2006 Jan-Feb;12(1):65-76. Epub 2005 Sep 2. Review.

Limb malformations and the human HOX genes.
Goodman FR.
Am J Med Genet. 2002 Oct 15;112(3):256-65. Review.