Regionalization of the nervous system 1

September 19, 2018
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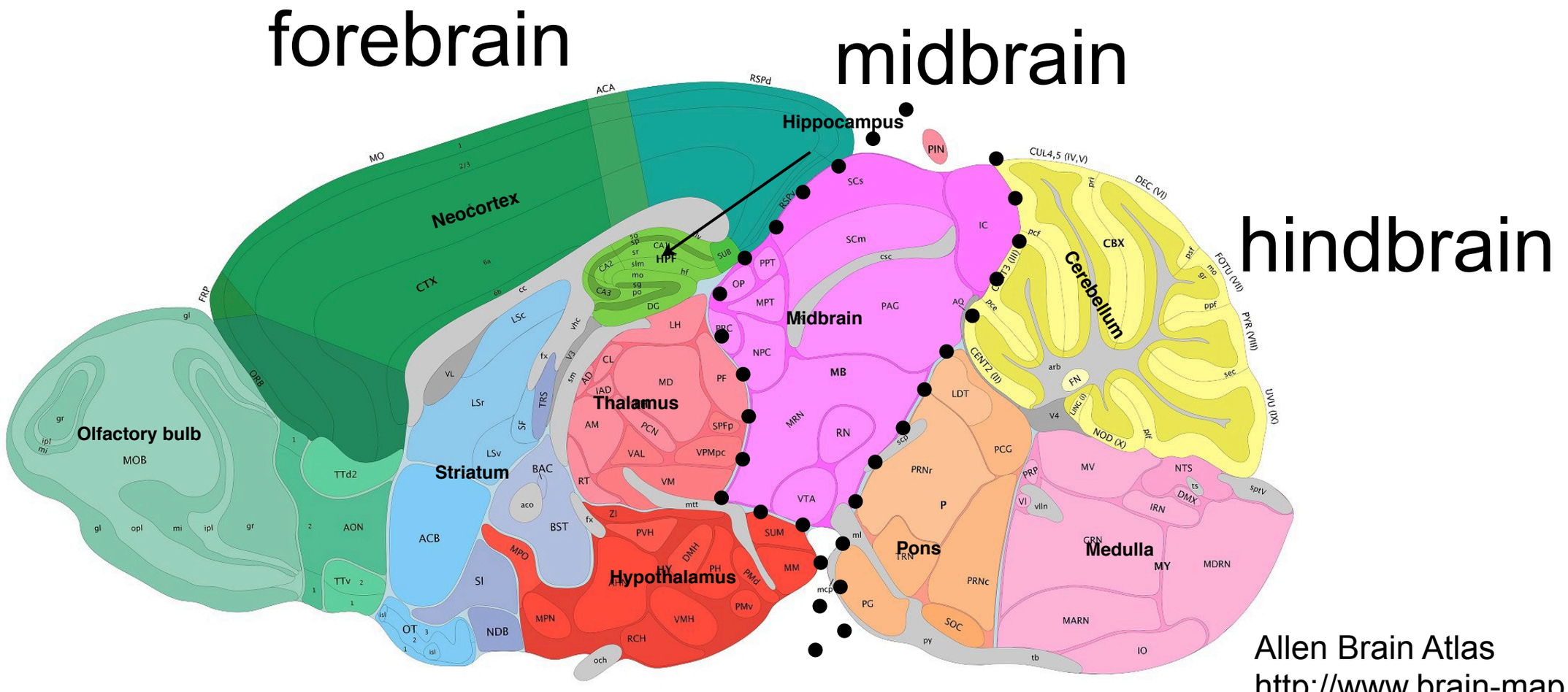
Complex organization of the adult brain



Allen Brain Atlas
http://www.brain-map.org/

How are all these regions established?

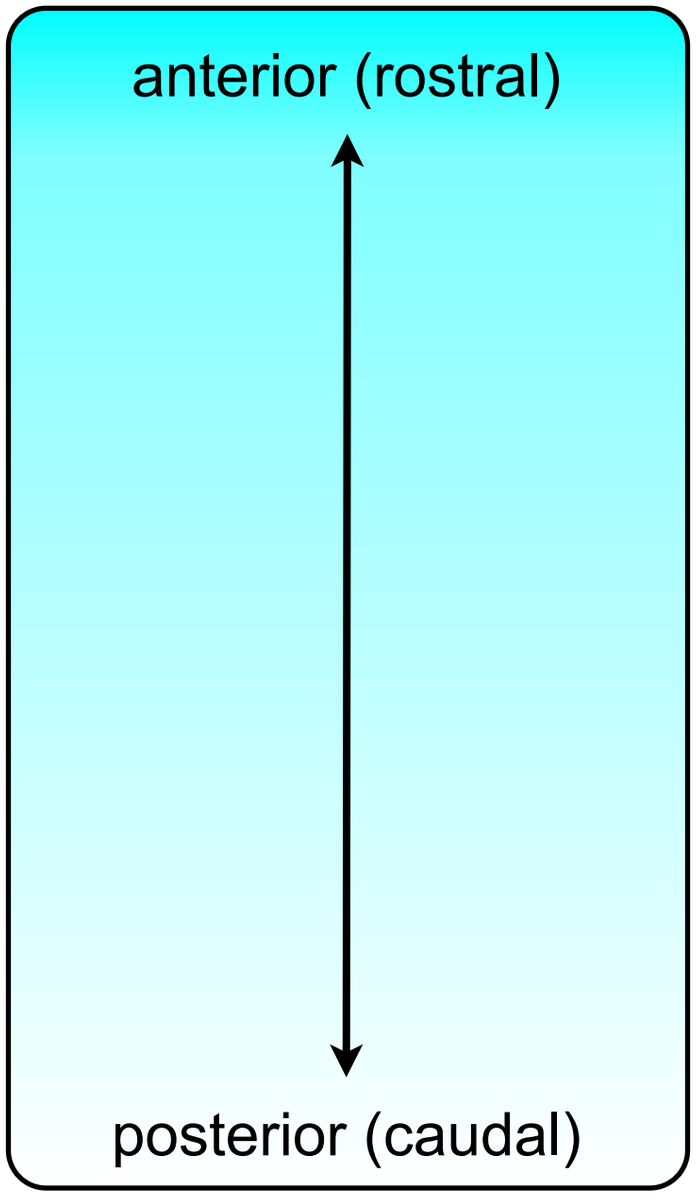
Regionalization generates diversity in cell types in the nervous system

Cells in early neural tissue acquire identity that is appropriate for their location ("positional identity"). This process is called regionalization.

Positional identity contributes to the generation of different types of neurons and glial cells.

Key molecular mechanisms of regionalization in the nervous system are:
-shared throughout evolution
  almost all molecules identified first by fly genetics
-used over and over throughout brain development as well as in the adult brain.

The neural plate is a two-dimensional structure



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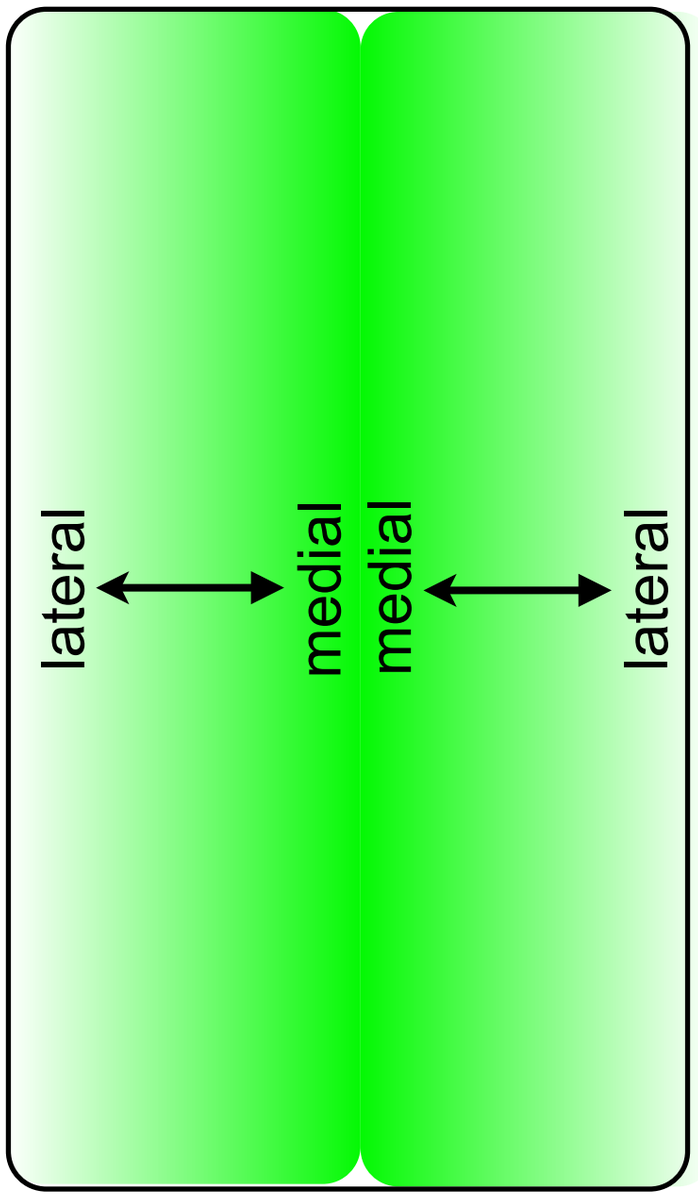
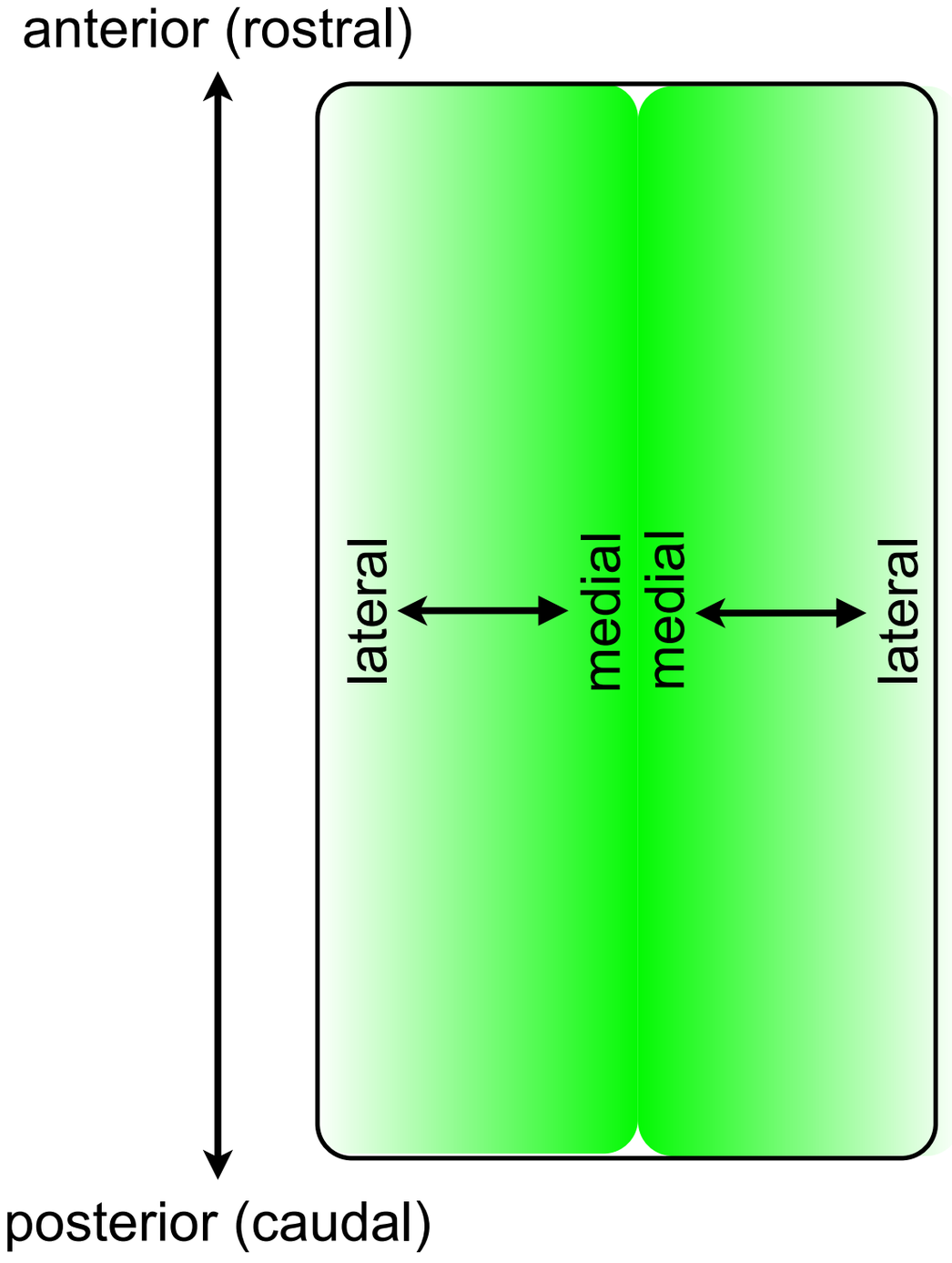
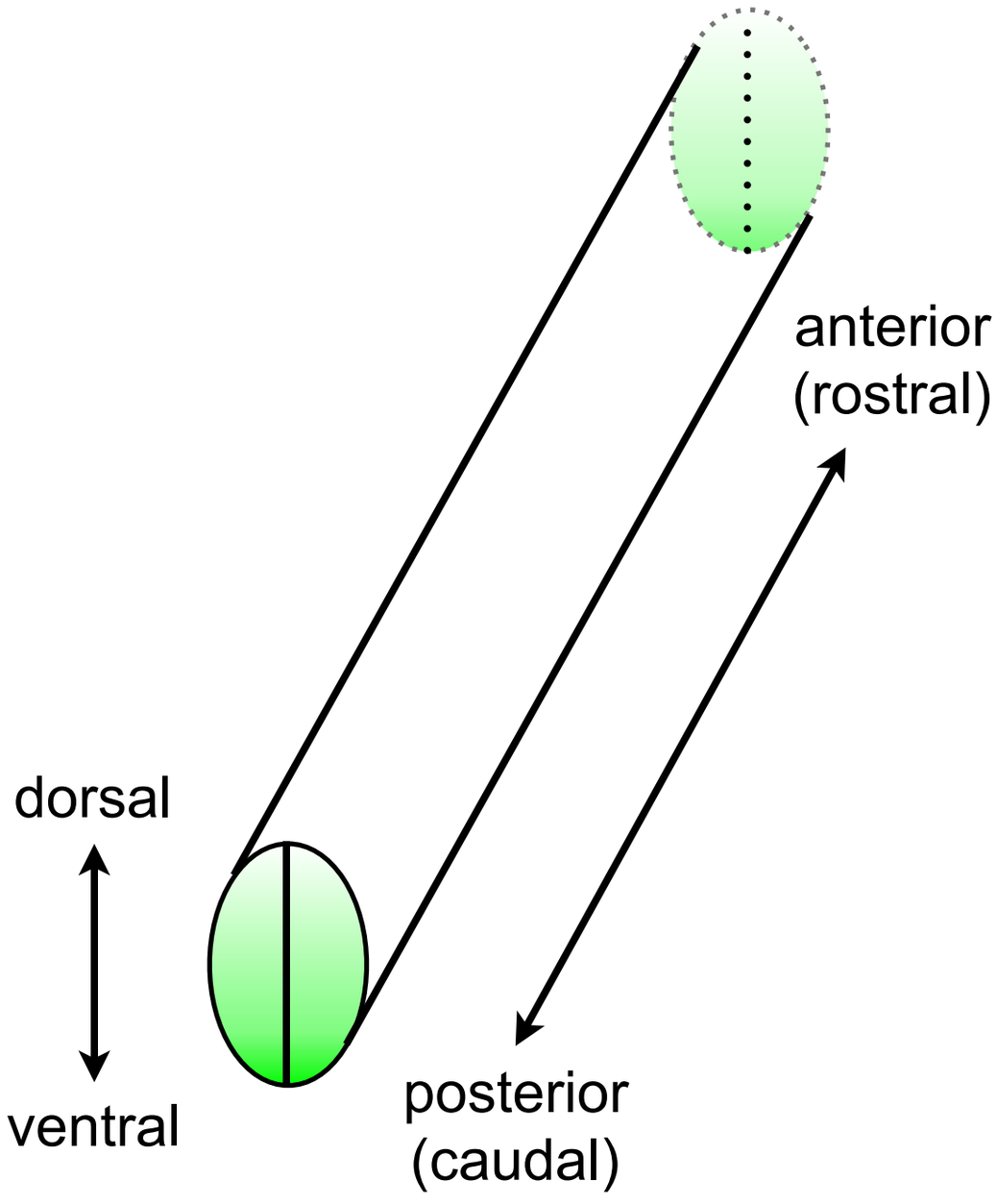
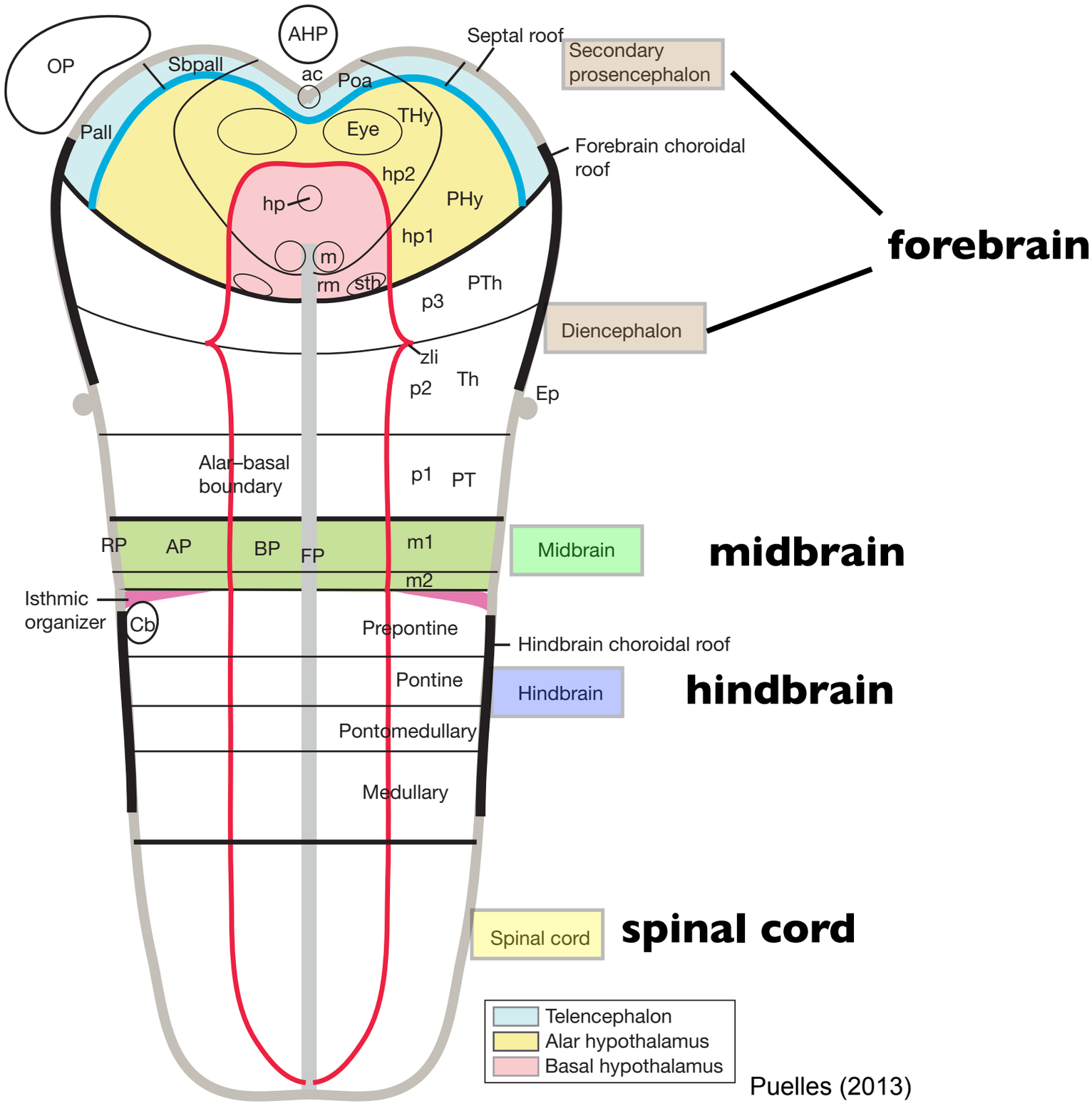


Medial-lateral axis becomes ventral-dorsal axis after neural tube closure



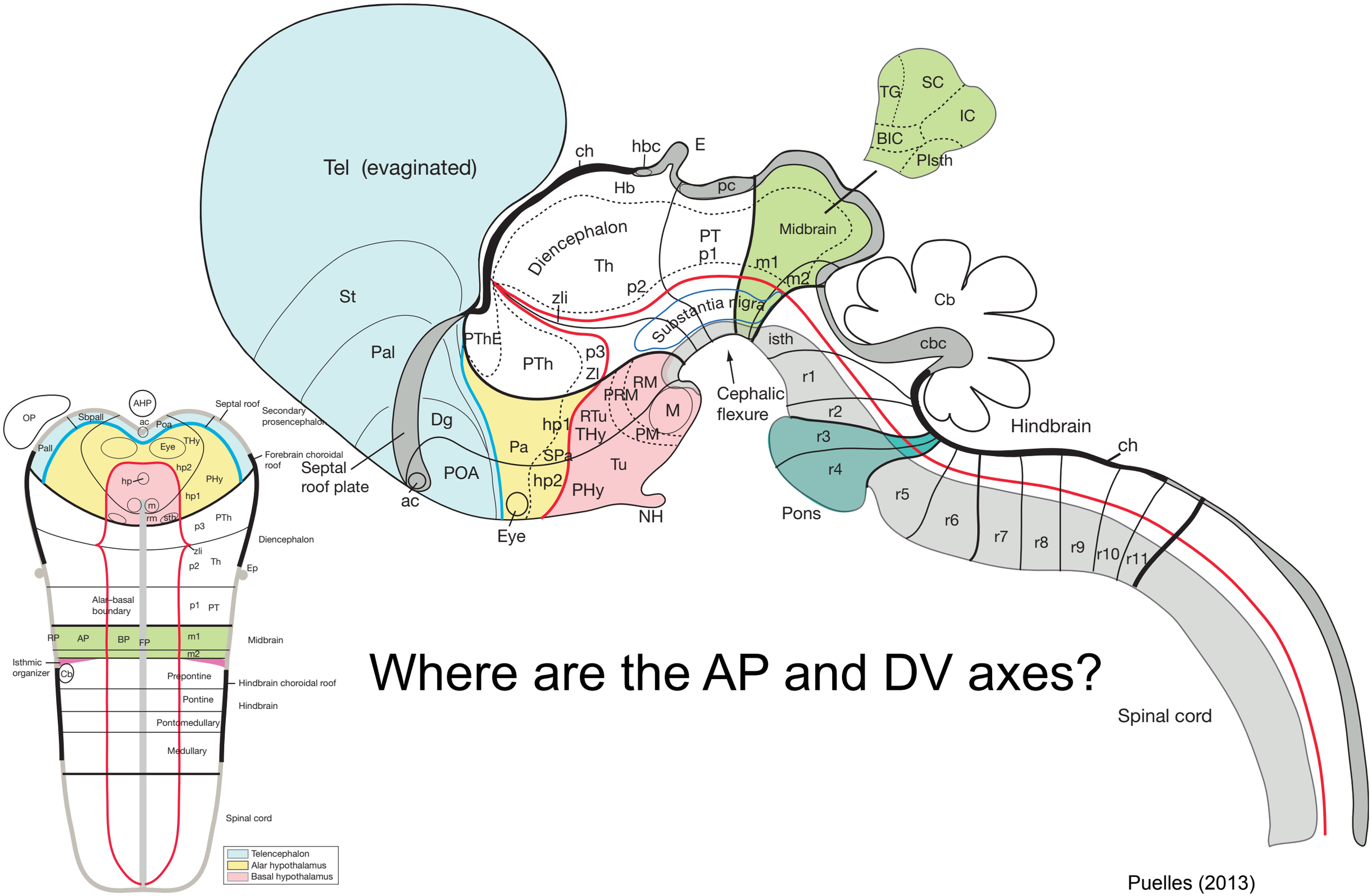


Fate map of the neural plate



Puelles (2013)

Subdivisions at later stages



Where are the AP and DV axes?

Puelles (2013)

Outline of lectures

Regionalization 1 (9/18/15)

neural induction and early regionalization along anterior-posterior (AP) axis
AP patterning in Drosophila embryos as a model of regionalization

Regionalization 2 (9/20/15)

AP patterning by secondary organizers
dorsoventral (DV) patterning

Research talk (10/2/15)

patterning, cell division and cell fate regulation in the mouse thalamus

Key concept 1

Early regionalization is linked to neural induction

-BMP inhibition induces anterior neural tissue.

-Posterior identity is induced independently of BMP inhibition.

Neural induction and regionalization in early embryos

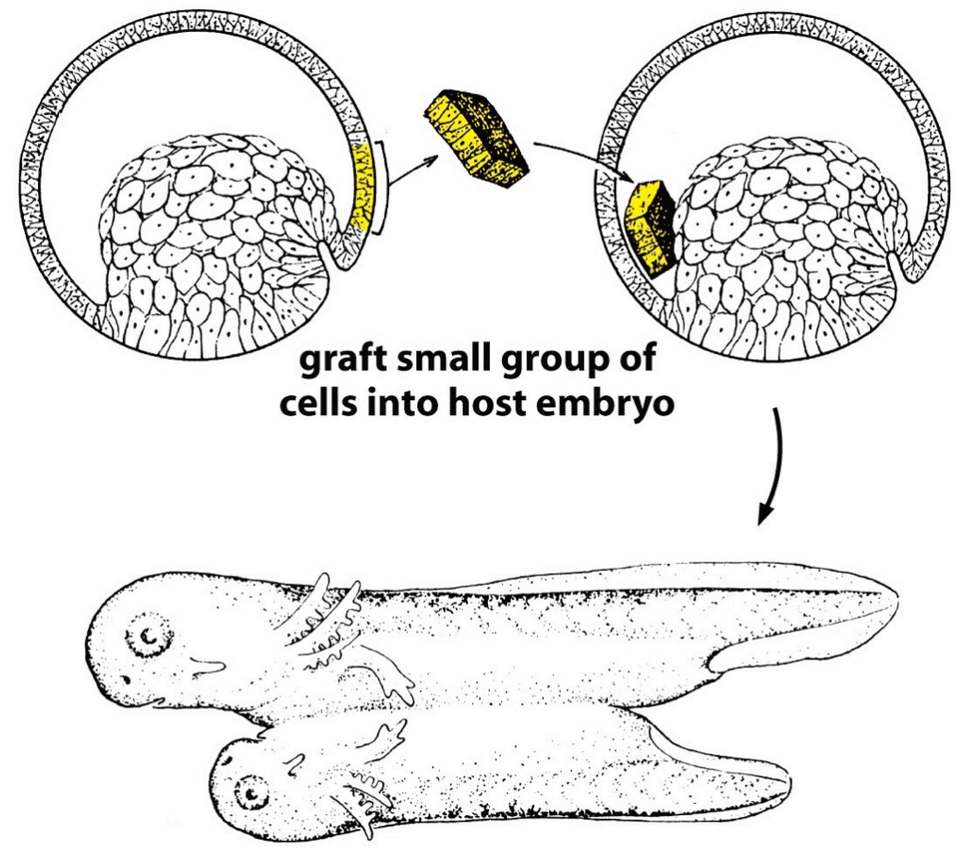


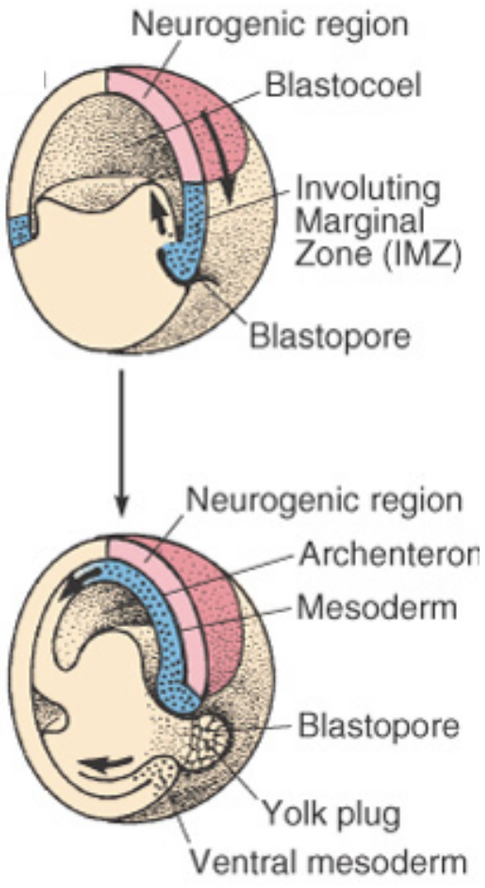
Figure 22-6b Molecular Biology of the Cell 5/e (© Garland Science 2008)

In Mangold/Spemann experiments, the transplanted organizer induced ectopic nervous system from cells not fated to form a neural plate.

The induced nervous system is appropriately patterned along its rostro-caudal (anterior-posterior) and dorso-ventral axes.

How are neural induction and regionalization mechanistically related?

Spemann organizer sequentially gives rise to axial mesoderm underneath the future neural tissue



Sanes, 2011

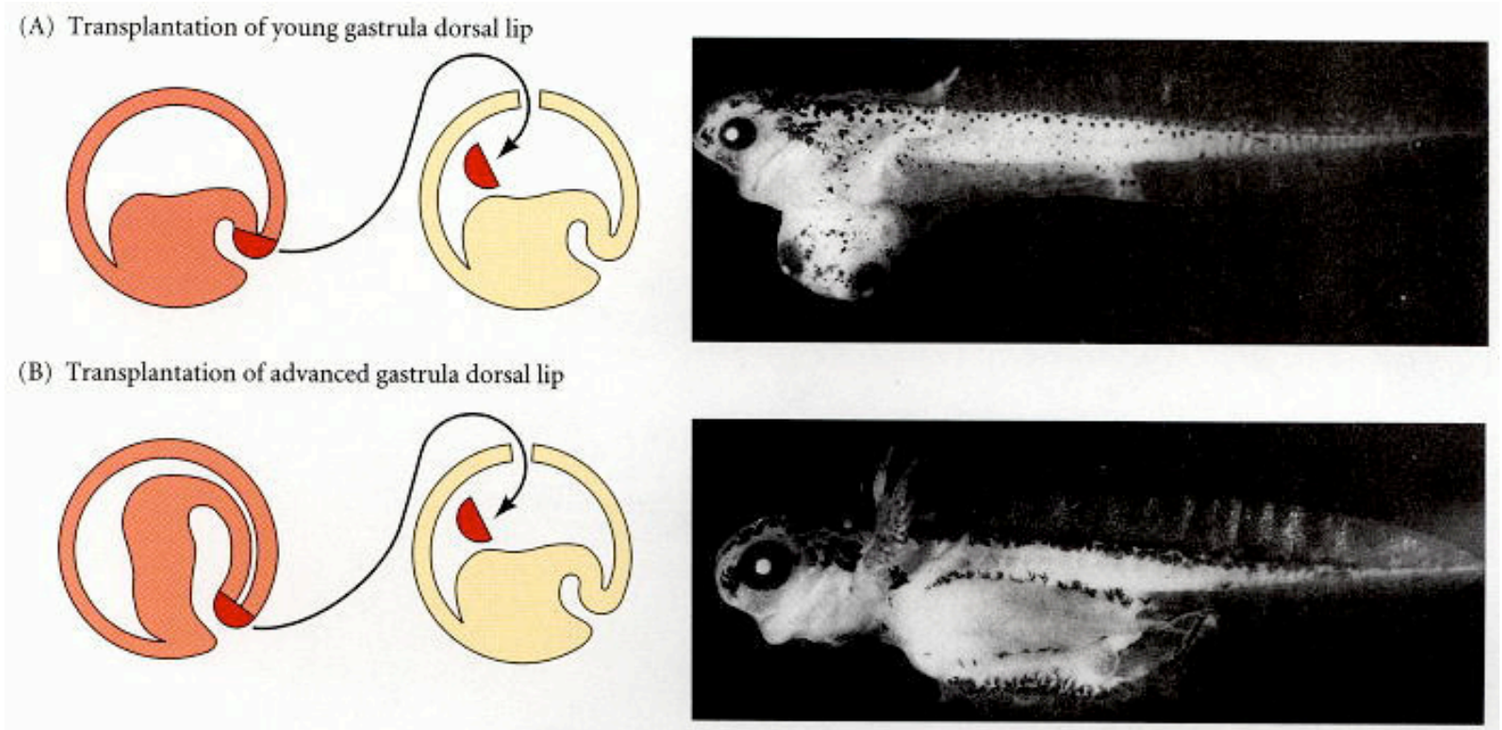
-Dorsal lip cells involute and form the **axial mesoderm** that underlie the presumptive neural plate.

- *prechordal mesoderm*: rostral, originated from **early** dorsal lip
- *notochordal mesoderm* (*notochord*): caudal, originated from **late** dorsal lip

-Like the dorsal lip itself, axial mesoderm can also induce neural tissue in amphibians, which led to an assumption that neural induction occurs largely via vertical signaling between the mesoderm and the overlying ectoderm.

-Do rostral and caudal axial mesoderm cells have different activity of inducing rostral vs caudal neural tissue?

Temporal specificity of induction

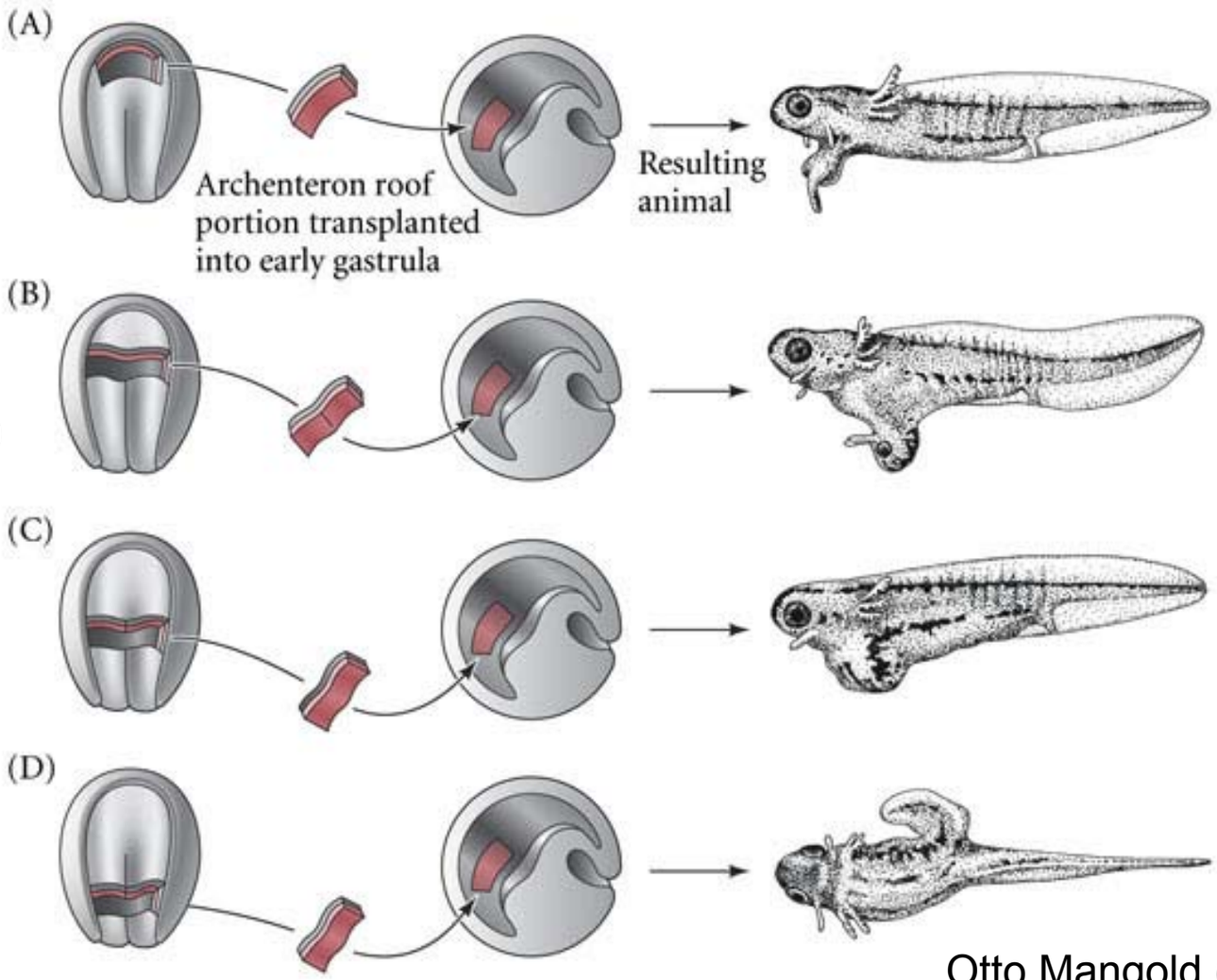


Young (early) dorsal lip induces a secondary head.

Older (late) dorsal lip induces a secondary trunk.

Regional specificity of induction

transplantation of different rostral-caudal parts of the archienteron roof into early gastrula



Otto Mangold (1933)

<u>induced tissue</u>

balancers and oral apparatus

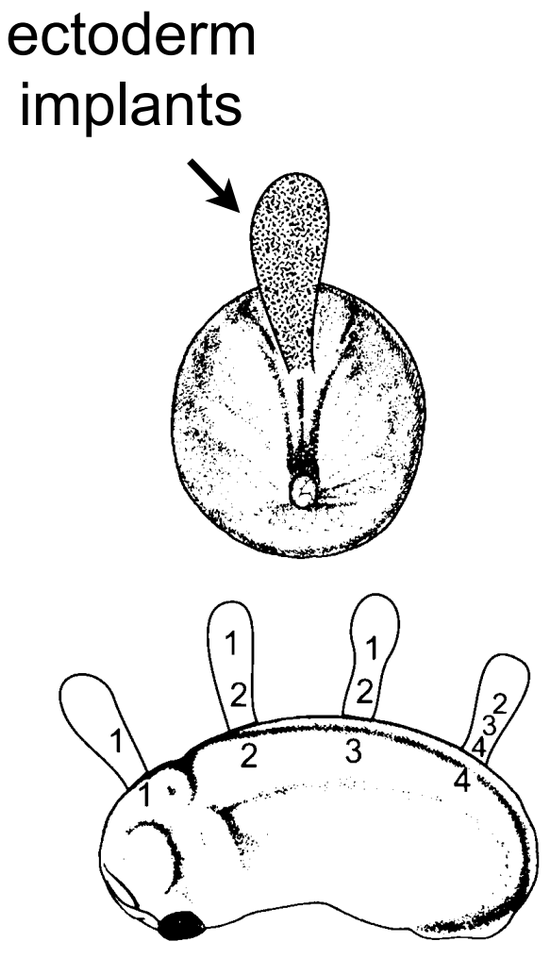
head structures (forebrain and midbrain)

hindbrain

spinal cord

This led Mangold to propose that there are distinct organizers that induce different regions of the neural tissue separately (**“head-trunk-tail organizer model”)**

Activation-transformation model



Niewkoop (1952)

Grafts of early ectodermal tissues were transplanted into different parts of the future neural tissue.

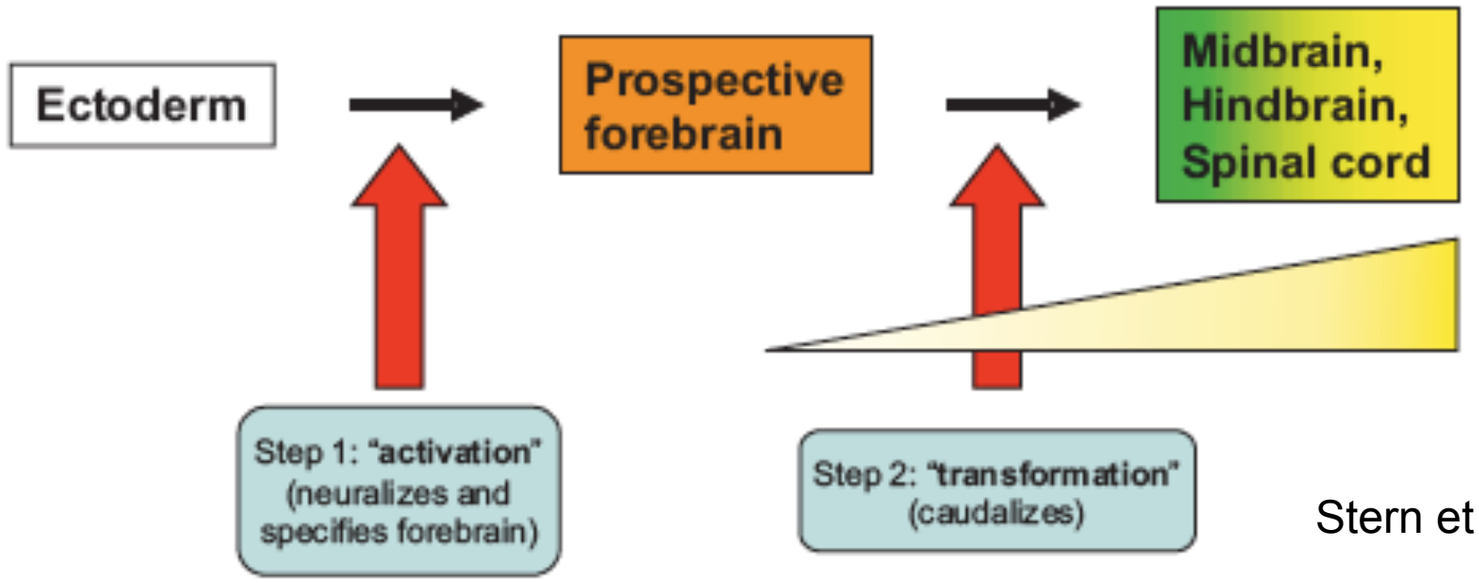
The proximal part included neural tissue, whereas the distal part included non-neural tissue

Within the induced neural tissue, the more distal part was always rostral whereas the more proximal part was always caudal

The level of the graft in the host always determined the regional character of the most caudal neural tissue in the graft.

Niewkoop and others proposed that the neural tissue is patterned by a gradient of a **transformer** that travels within the plane of the neural plate and induces different neural fate in a dose-dependent manner such that forebrain, midbrain, hindbrain and spinal cord form at increasing levels of this transformer (**activation-transformation model**).

Activation-transformation model



Stern et al. 2006

What is the molecular basis for this classic model?

-We now know that “activation” occurs via inhibition of BMP signaling.

-Dissociated animal cap cells will generate neural cells with **anterior** identity (in the absence of exogenous BMP). Is this the default positional identity? If so, what molecules are responsible for “transformation”? Where are they expressed?

-Are there endogenous inhibitors of transformer activities that counteract such activities? If so, where are they expressed?

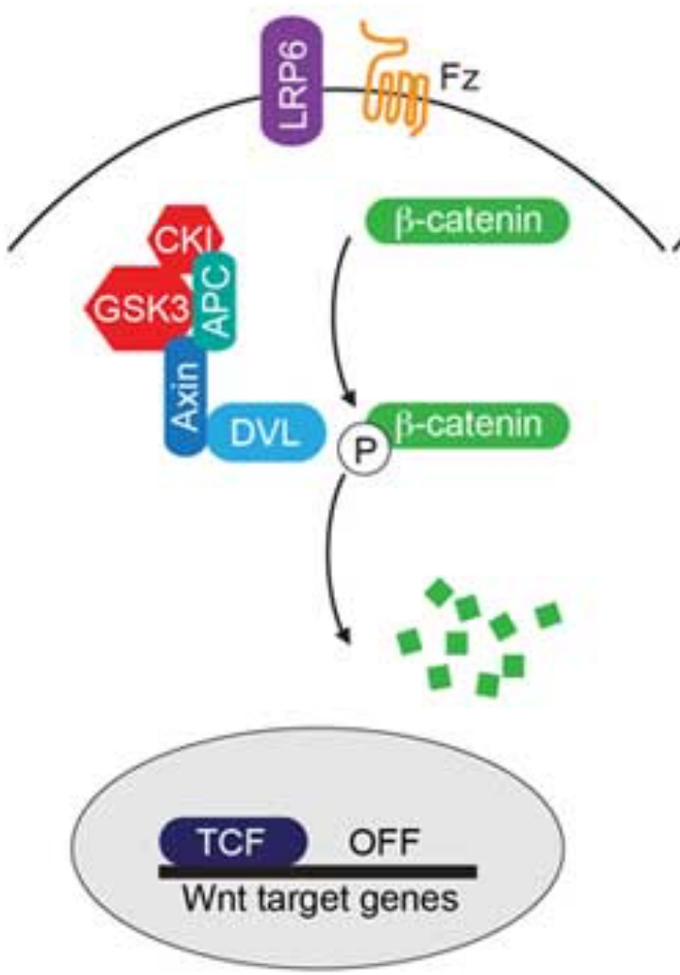
-Molecules responsible for caudalizing activity include Wnts, fibroblast growth factors (FGFs) and retinoic acid (RA).

Wnt signaling pathway

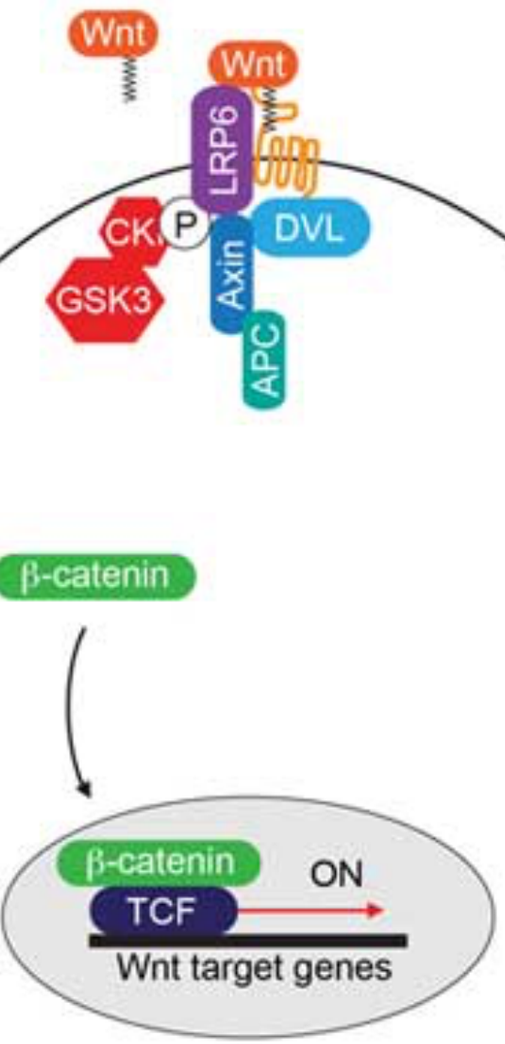
Wnts: evolutionarily conserved secreted proteins
-19 Wnt genes in human genome
-multiple signaling pathways depending on the cellular context and receptors

<u>"canonical" pathway</u>

Wnt signaling OFF



Wnt signaling ON



Wnt signaling OFF:

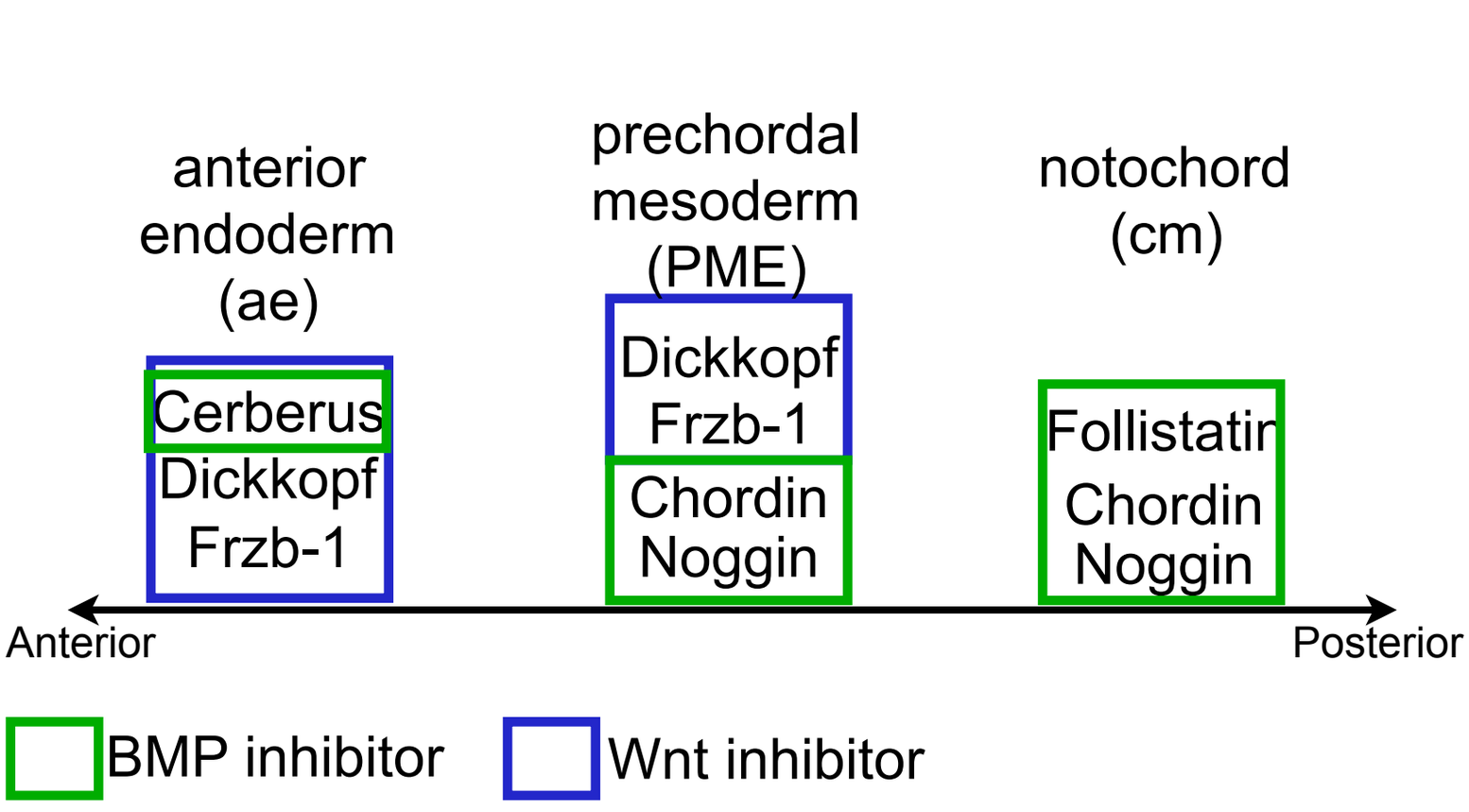
•β-catenin is targeted for degradation by a destruction complex.

Wnt signaling ON:

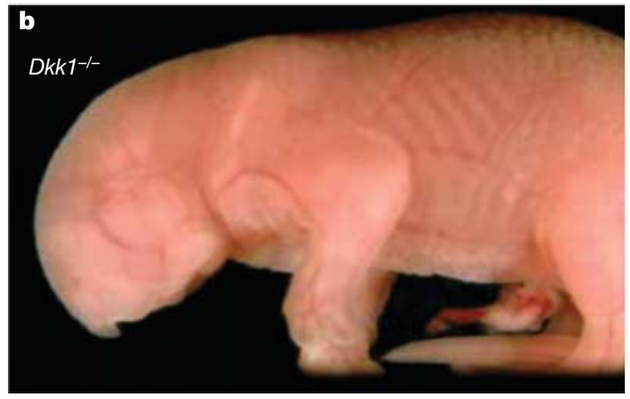
•Binding of Wnt to Frizzled and LRP6 leads to inhibition of β-catenin degradation.
•Stabilized β-catenin translocates to the nucleus and interacts with TCF/Lef1 family of HMG-box containing transcription factors to activate target gene transcription.

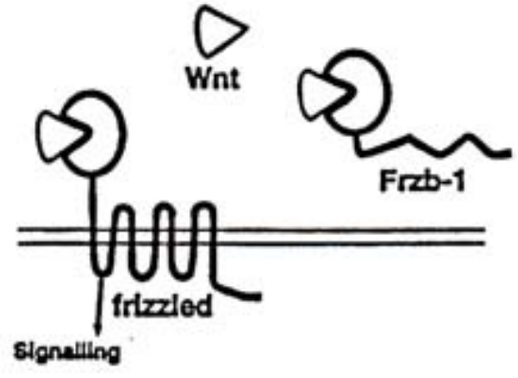
http://wormbook.org/chapters/www_wntsignaling.2/wntsignal.html

Tissues underlying the anterior brain express Wnt inhibitors

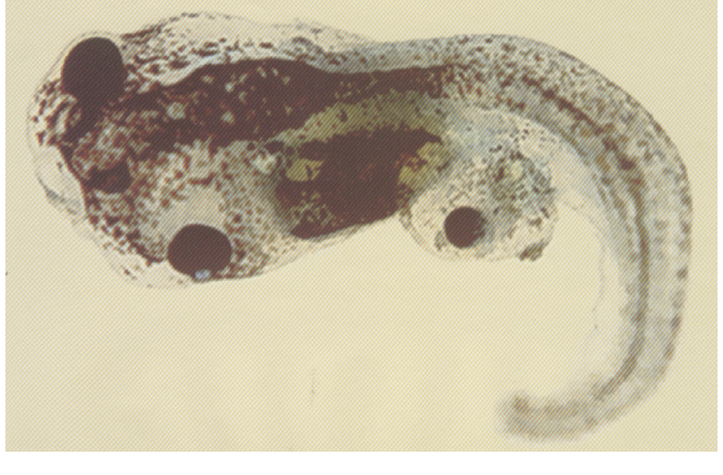


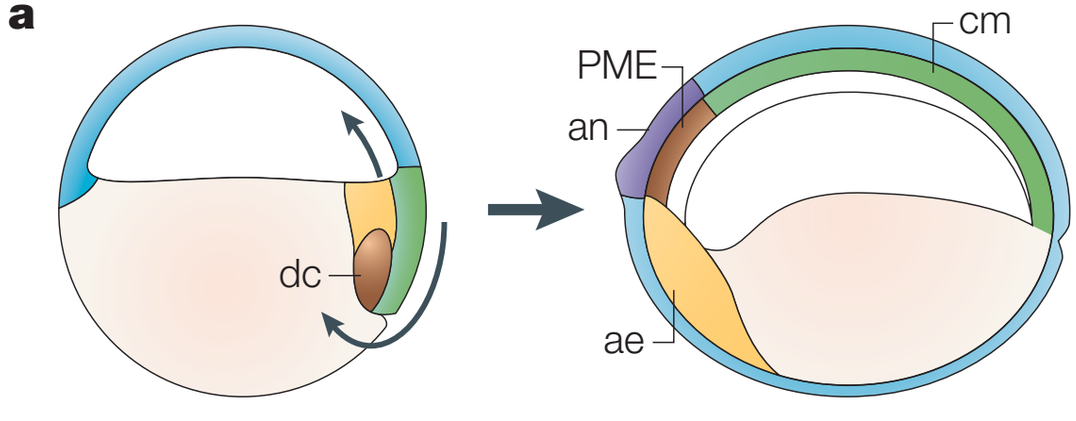
Dkk1 ko mouse





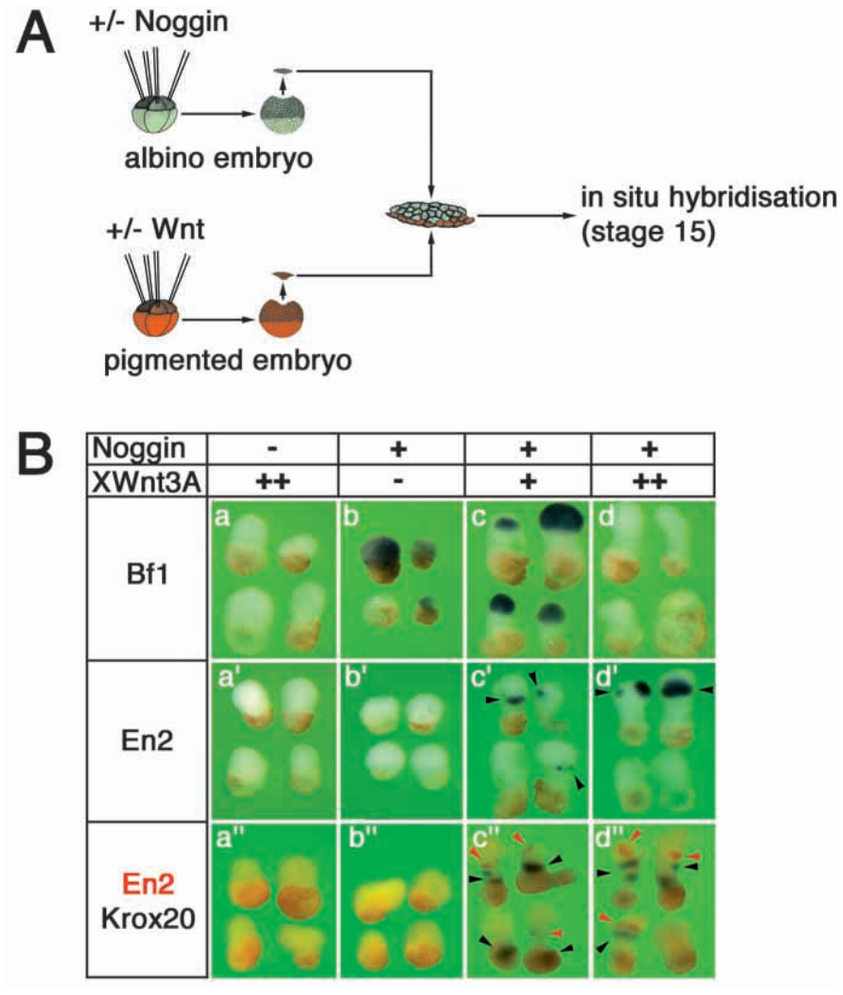
Over-expression of Cerberus generates an ectopic head



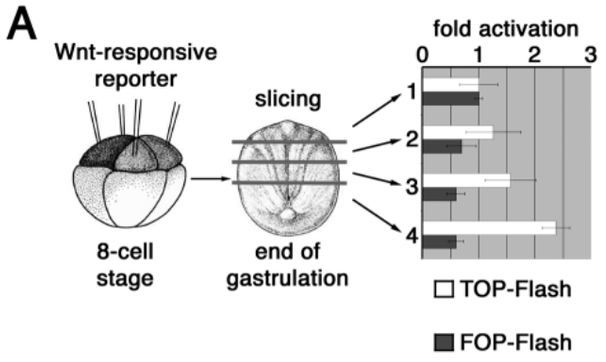


Graded Wnt/β-catenin signaling acts as transforming activity

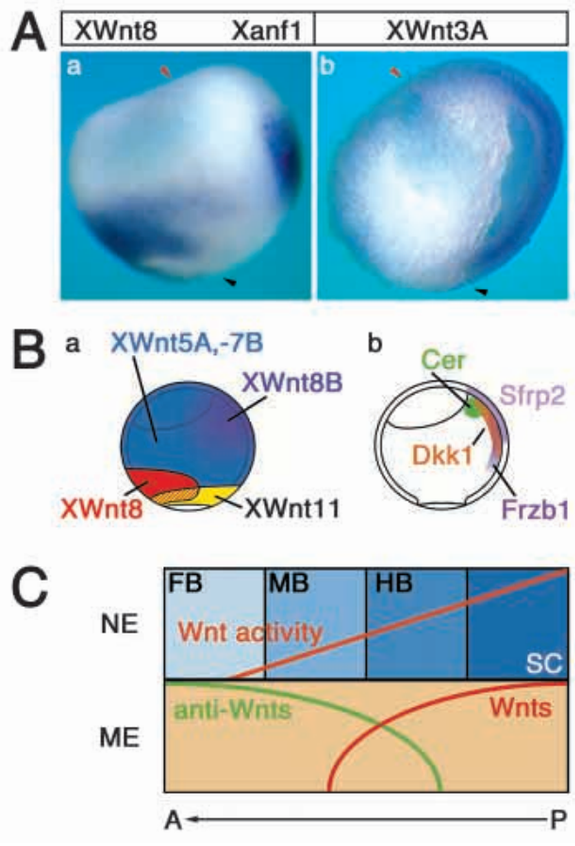
-In vivo and in vitro Wnt over-expression in Xenopus, zebrafish, chick and mouse results in caudalizatoin of the neural tissue.



-Late Xenopus gastrula shows an AP gradient of Wnt/β-catenin signaling



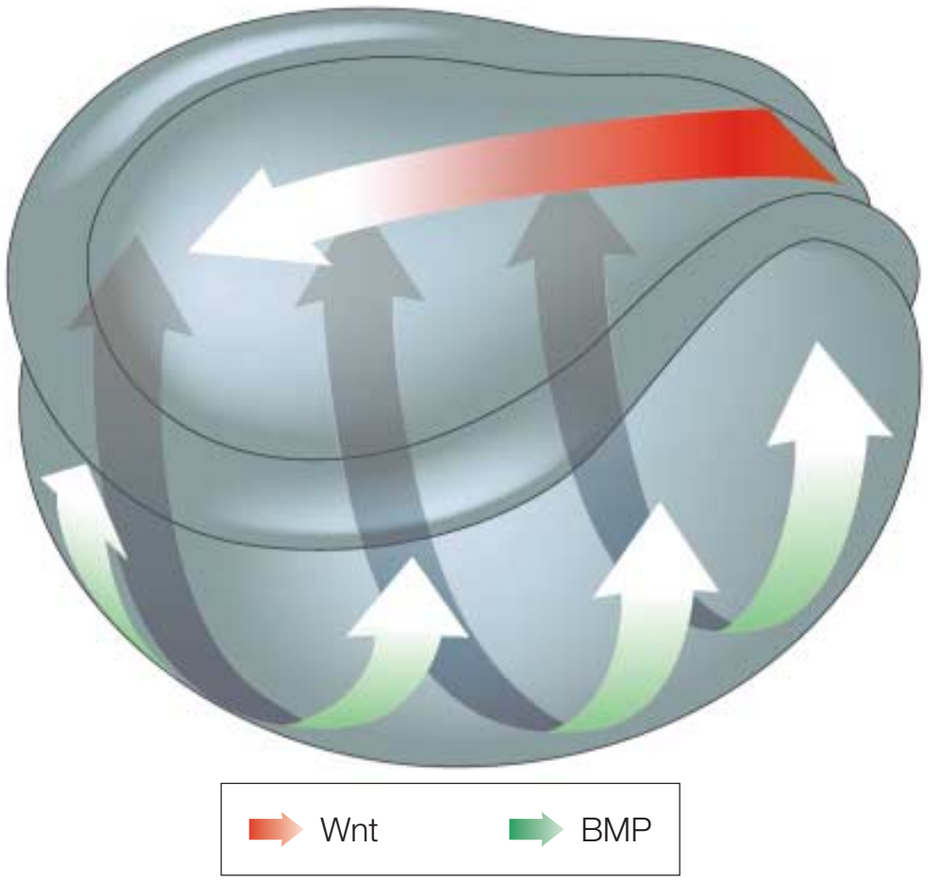
-Wnt antagonists are expressed in the anterior part of the gastrula



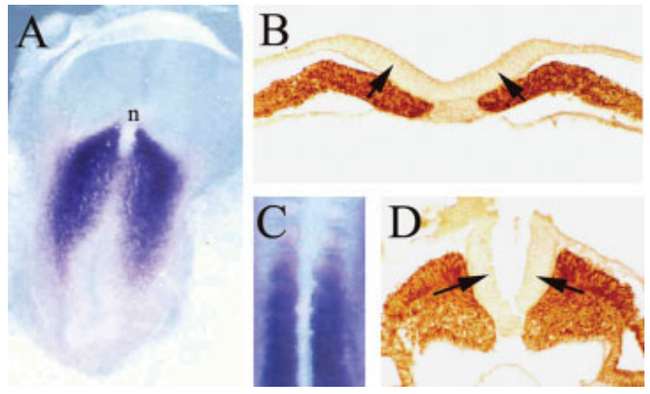
Kiecker and Niehrs (2001)

Transcription factors are differentially expressed in rostral and caudal neural tissue.

Early signals come from outside of the nervous system and establish gross AP patterns

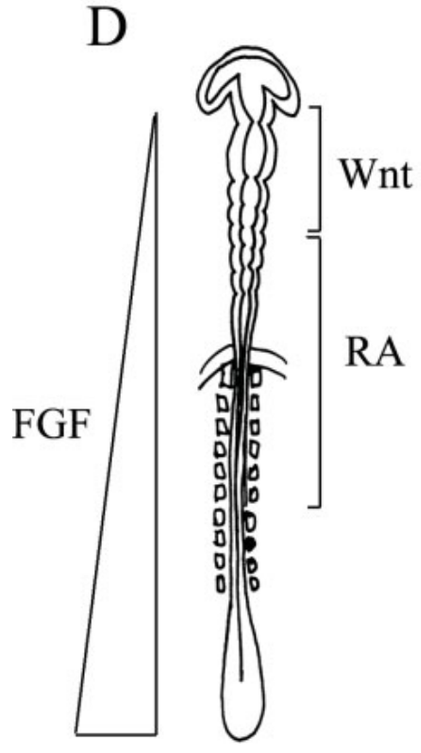


Caudalizing factors (Wnts, RA, FGFs) are generally produced by **paraxial** mesoderm (somites), not axial mesoderm (descendants of the organizer), and they do not induce neural tissue on their own.



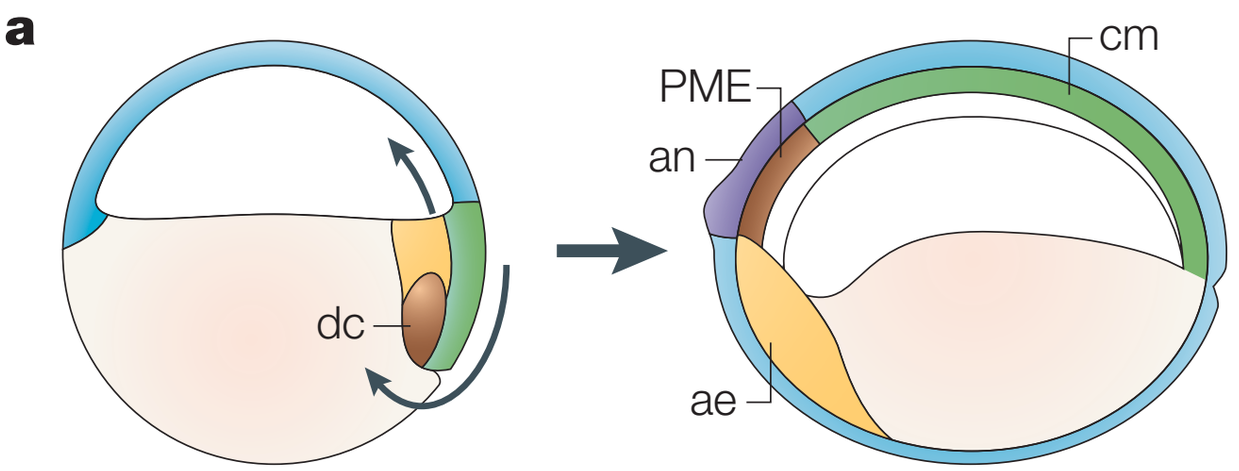
expression of RA-synthesizing enzyme, Radlh2 in chick somites

Different caudalizing factors are responsible for patterning at different AP levels in the nervous system

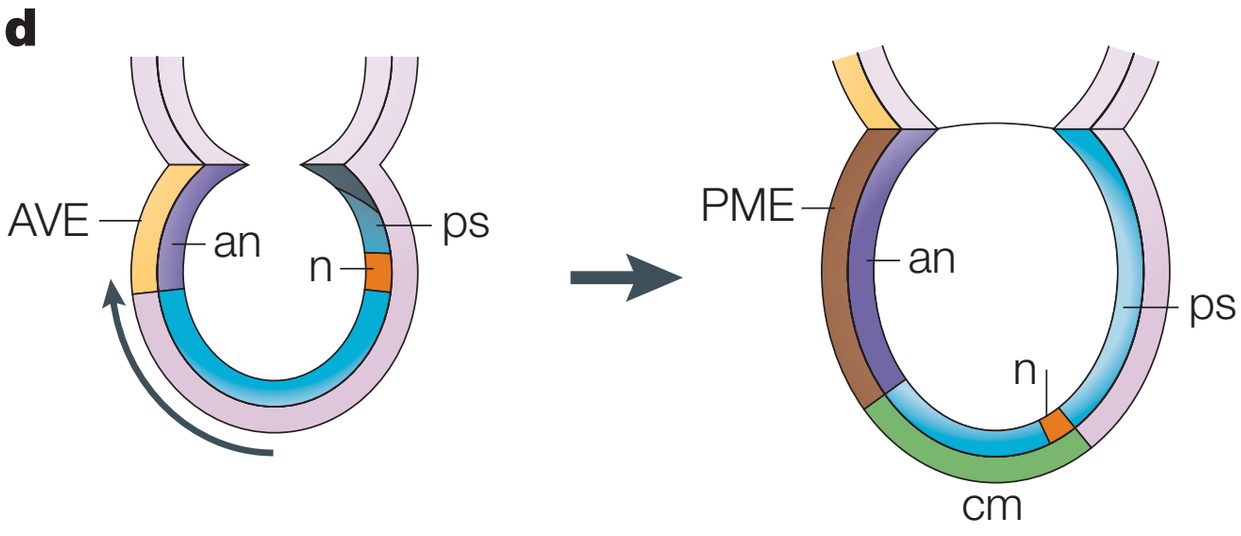


Mechanisms for early regionalization are similar in different vertebrate species

Xenopus



Mouse



Cylinder-shaped mouse embryo becomes asymmetric by the formation of anterior visceral endoderm (AVE). Like the amphibian anterior endoderm, AVE produces Cerberus, a Wnt inhibitor.

Formation of AVE triggers the formation of primitive streak (ps) on the opposite (caudal) side. The primitive streak is equivalent to the amphibian blastopore, and produces Fgf, Wnt and Nodal.

Node (or Hansen's node) is formed at the anterior end of primitive streak. Node is equivalent to the amphibian organizer, and produces chordin. Derivatives of the node (cm and PME) also produce BMP inhibitors like chordin.

AVE cannot induce neural tissue by itself, but inhibits caudalization of the neural tissue by blocking Wnt, BMP and Nodal pathways.

Summary of part 1

Early regionalization is linked to neural induction

-BMP inhibition induces anterior neural tissue (default neural fate).

-Posterior identity is induced independently of BMP inhibition.
- •caudalizing (transforming) activity: Wnts, FGFs, RA
- •Inhibitors of Wnt proteins are expressed underneath the anterior neural tissue and prevent the neural tissue from becoming caudalized.

-Mechanisms of early regionalization are similar between vertebrate species.

Key concept 2

Signaling pathways and molecules involved in regionalization of the vertebrate nervous system were initially discovered in fly genetics

-Signaling pathway
- •cascade of gene regulation
- •graded expression of transcription factors forms discrete boundaries within embryos

-Molecules
- •*Hox* genes (encodes a family of transcription factors) are involved in identity of specific body segments.
- •secreted signaling molecules and their receptors

Embryonic AP patterning in Drosophila melanogaster

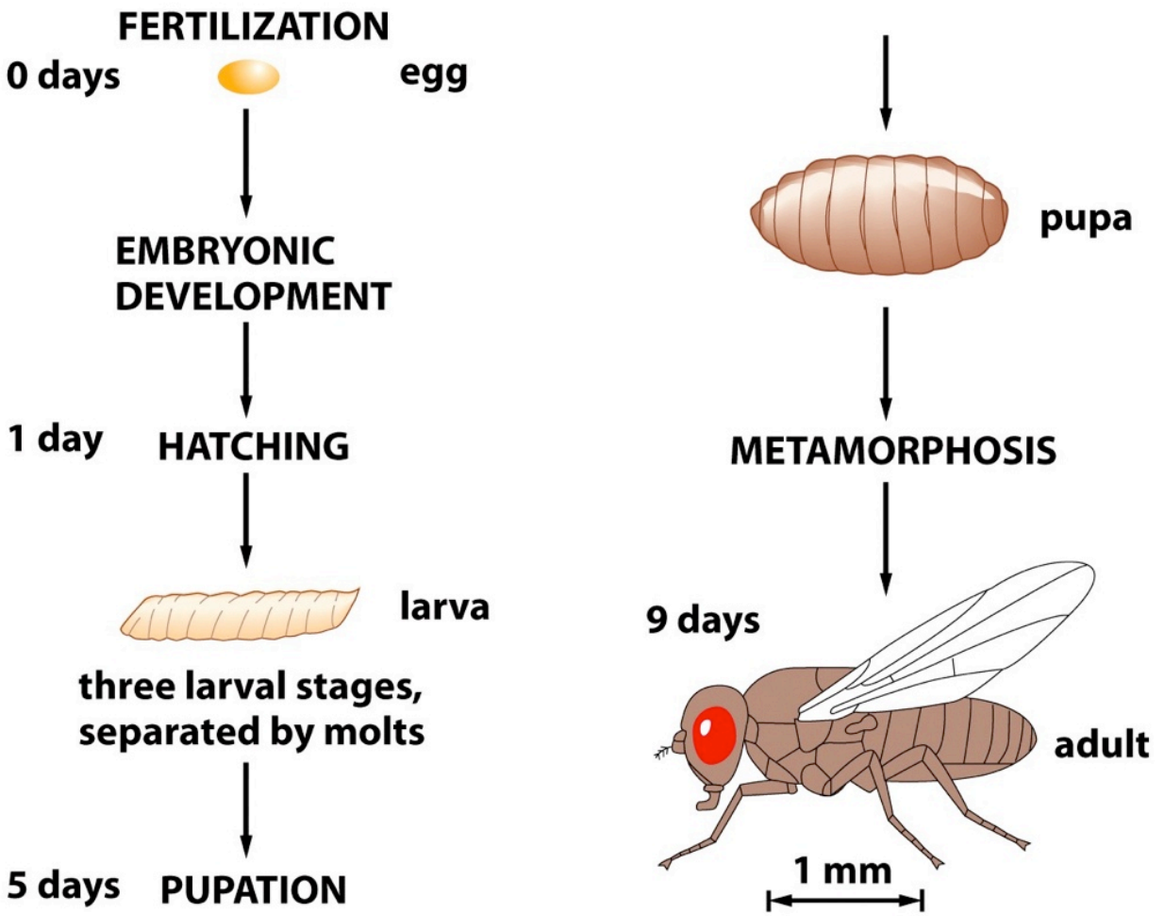


Figure 22-25 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The fly consists of a head (with mouth, eyes, antennae), three thoracic segments (T1-3) and 8-9 abdominal segments (A1-9)

The segmentation starts to develop in early embryos

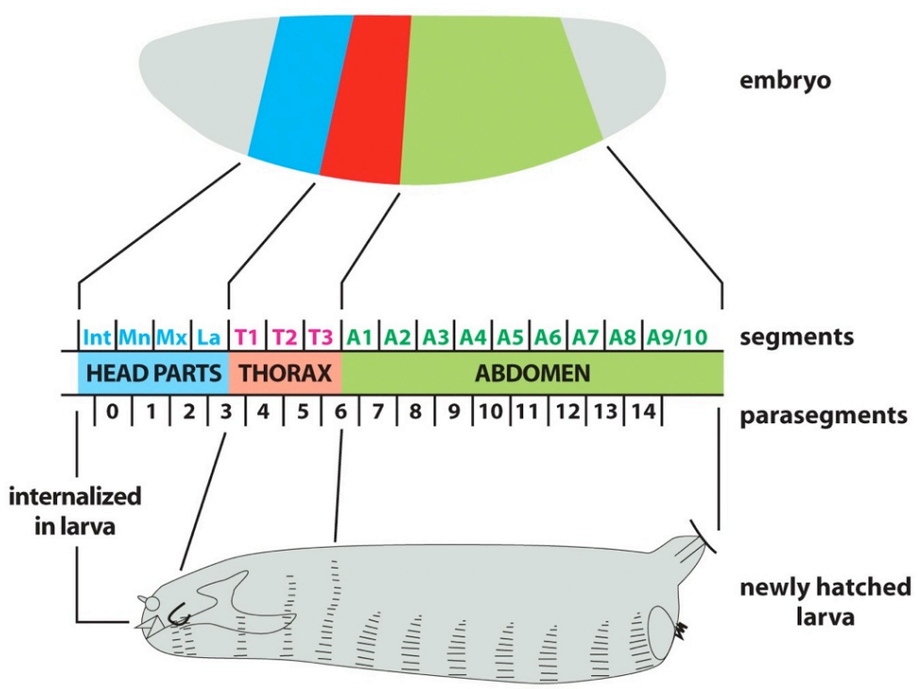


Figure 22-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Genetic mutant screening identified many genes involved in AP patterning in fly embryos

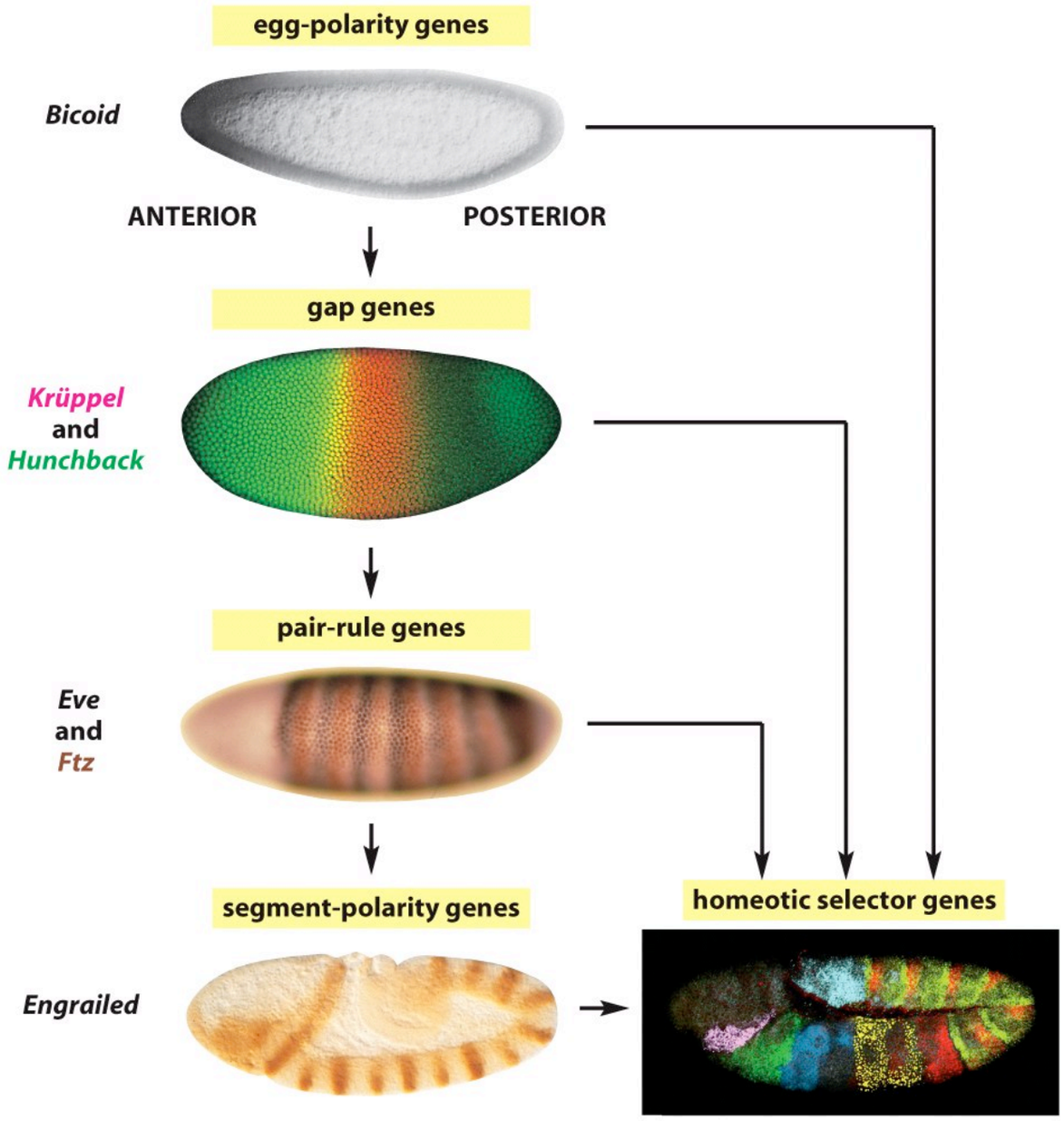


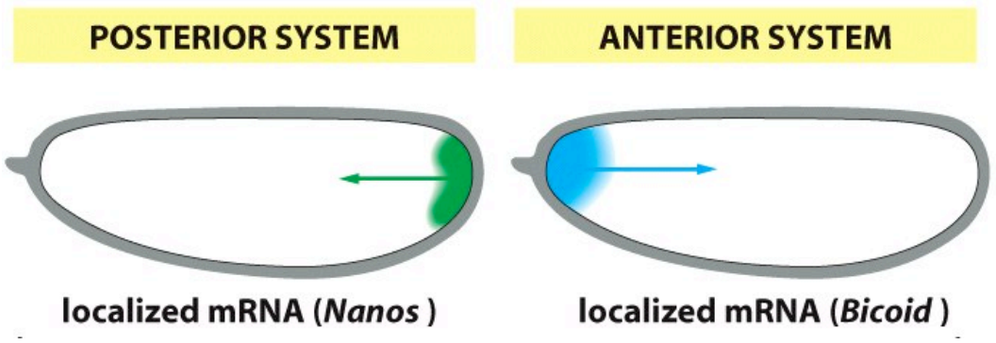
Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

-Genetic screens pioneered by Nüsslein-Volhard and Wiechaus in the 1980s identified a hierarchy of genes that establish anterior-posterior polarity of Drosophila embryos and divide the embryo into a specific number of segments with different identities.

-Basic ideas of the identified gene regulatory cascade apply to many other aspects of animal development, including the regionalization of the vertebrate nervous system.

-Many genes identified in this screen have vertebrate homologs that are important in the patterning of the neural tissue.

Polarization starts in unfertilized oocytes



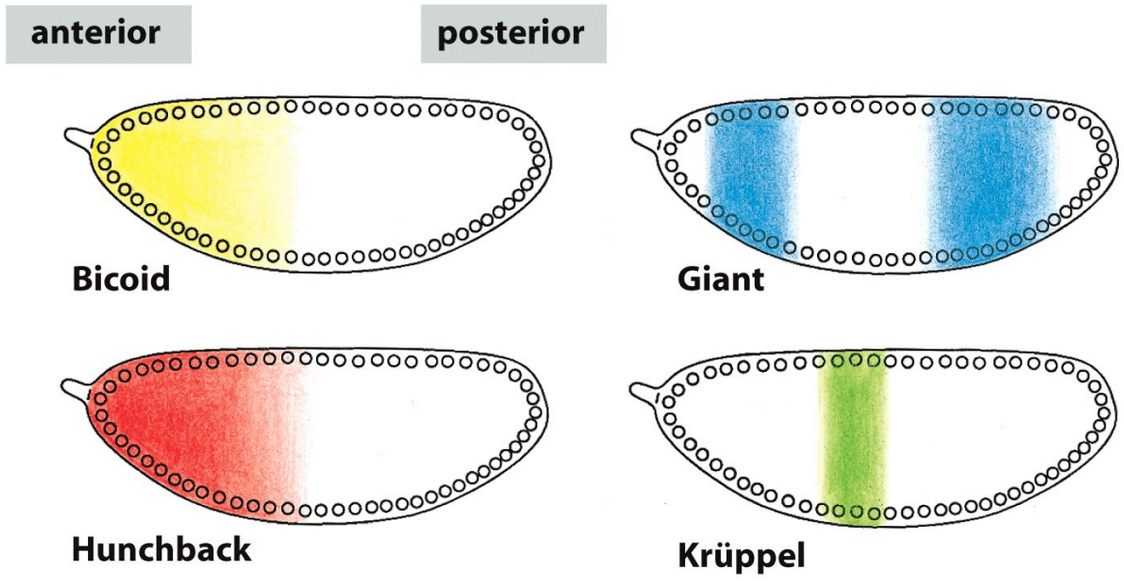


Figure 7-53 Molecular Biology of the Cell 5/e (© Garland Science 2008)

bicoid and *nanos* mRNAs are near the anterior and posterior end of the oocyte, respectively (**egg-polarity genes**)

Bicoid protein diffuses and forms a concentration gradient, regulating the graded expression of Hunchback

Hunchback, Krüppel and Giant are products of the **gap genes**, which mark out coarse subdivisions of the embryo

Pair-rule genes are required for alternative body segments

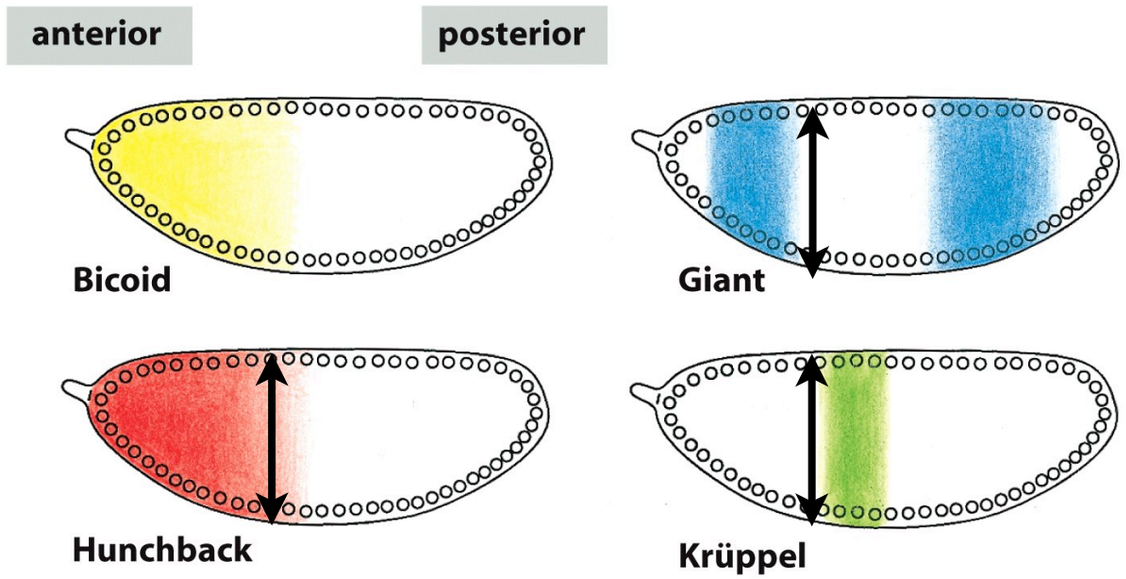


Figure 7-53 Molecular Biology of the Cell 5/e (© Garland Science 2008)

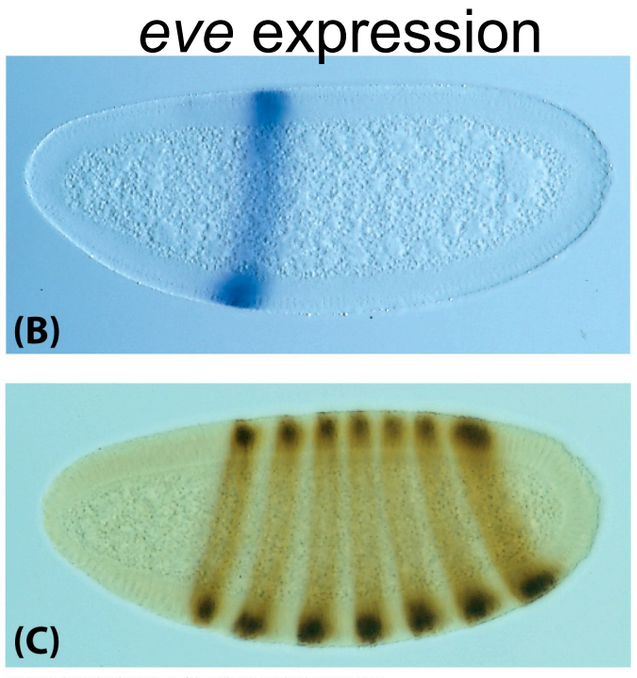


Figure 7-55bc Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Expression of *even-skipped (eve)* and *fushi tarazu (ftz)* are under the combinatorial regulation of gap genes.
- Discrete domains of gene expression are formed by a combination of upstream regulatory mechanisms.

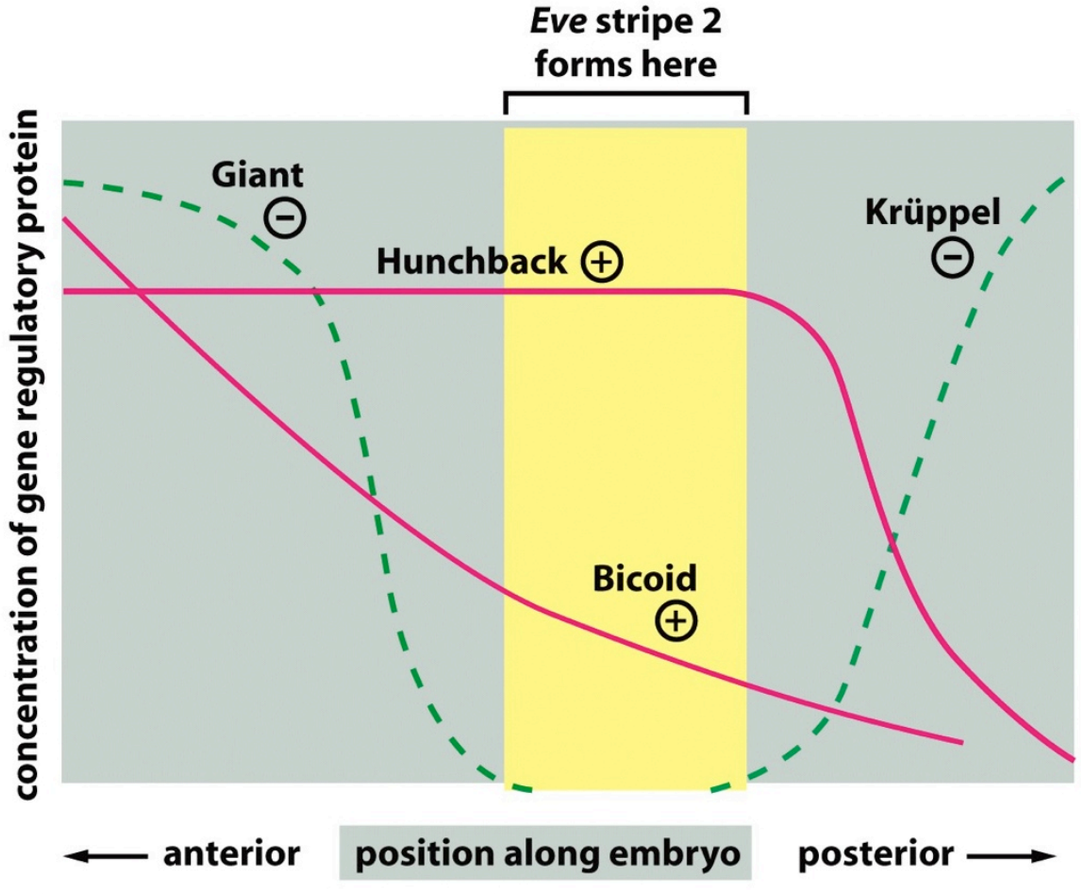


Figure 7-57 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Segment polarity genes organize the AP pattern of individual segment

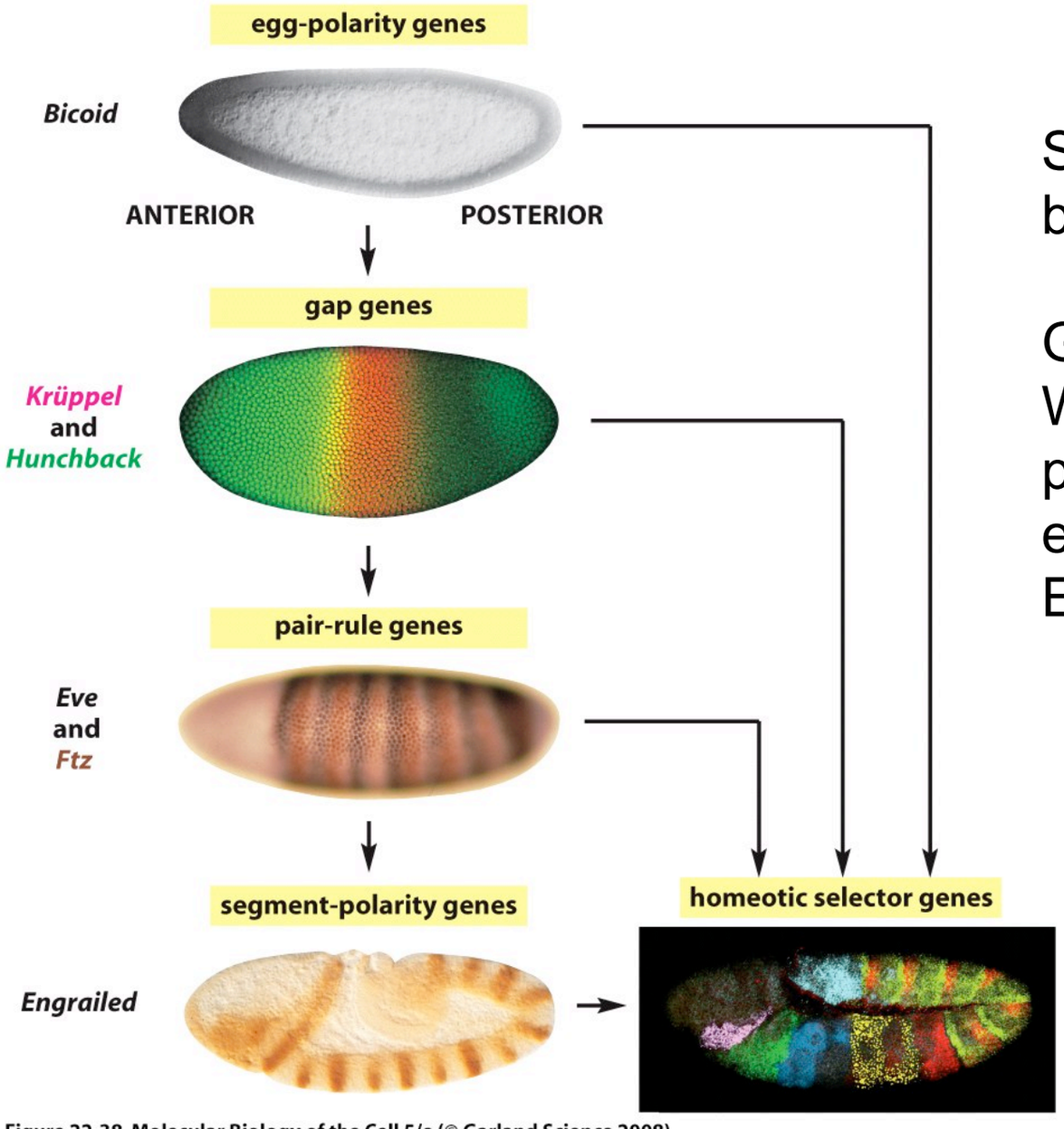
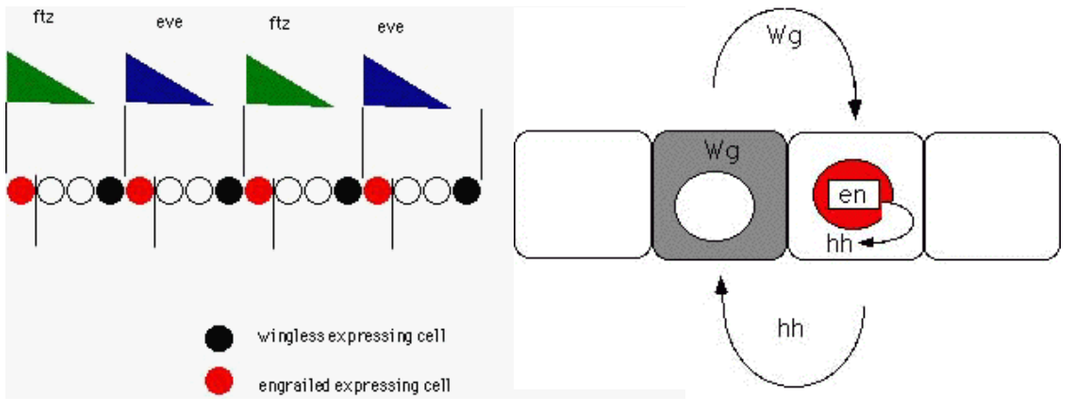


Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Segment polarity genes stabilize boundary between segments.

Genes encoding two secreted proteins, Wingless and Hedgehog, are segment polarity genes. They promote each other's expression as well as a transcription factor Engrailed.



Homeotic selector genes are required for the identity of each segment

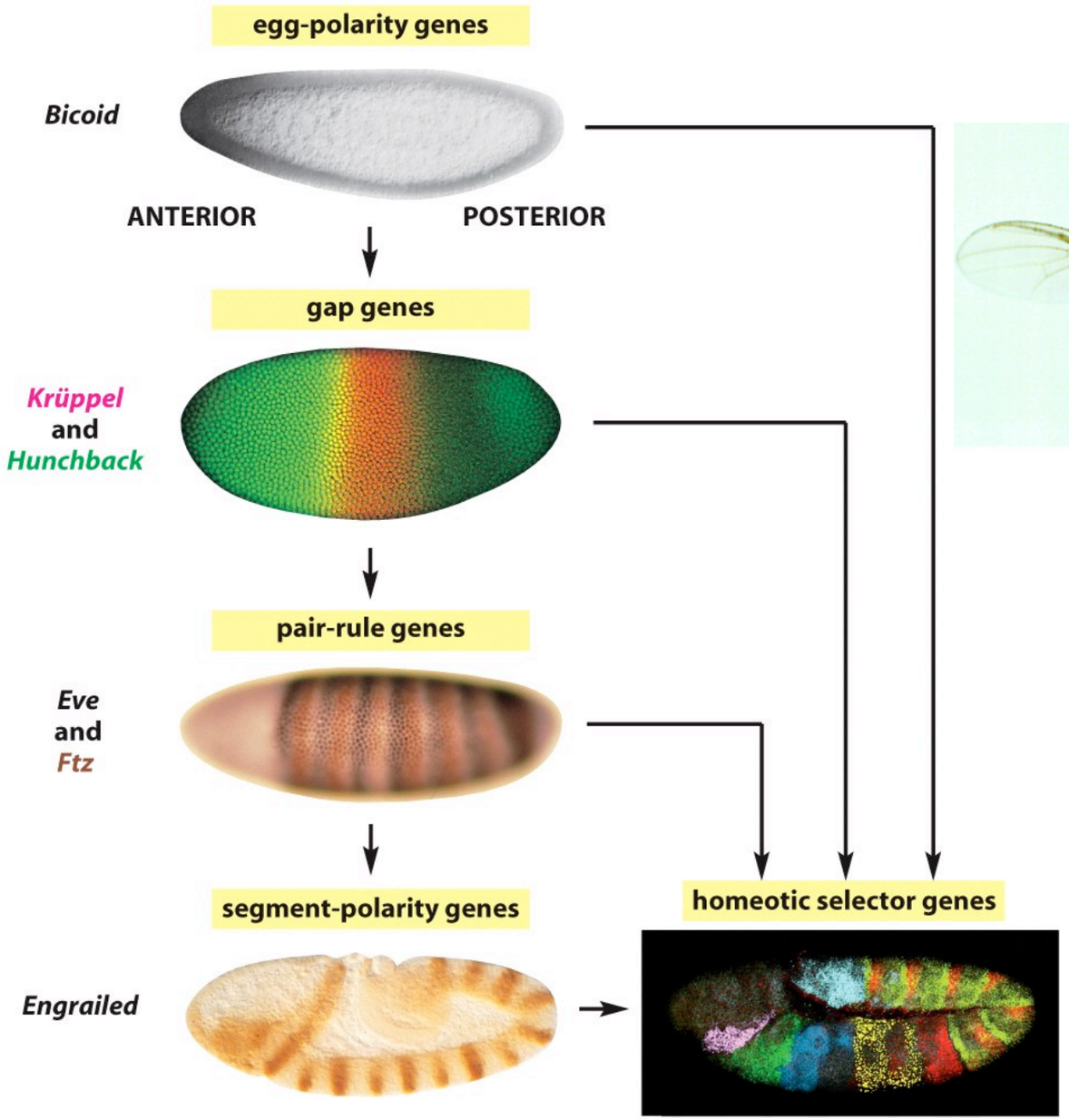


Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

extra pair of wings

Figure 22-42 Molecular Biology of the Cell 5/e (© Garland Science 2008)

legs sprout from the head instead of antennae

Antennapedia

Mutations that transform parts of the body into structures appropriate for other positions are called **homeotic mutations**

Homeotic selector genes code for DNA-binding proteins

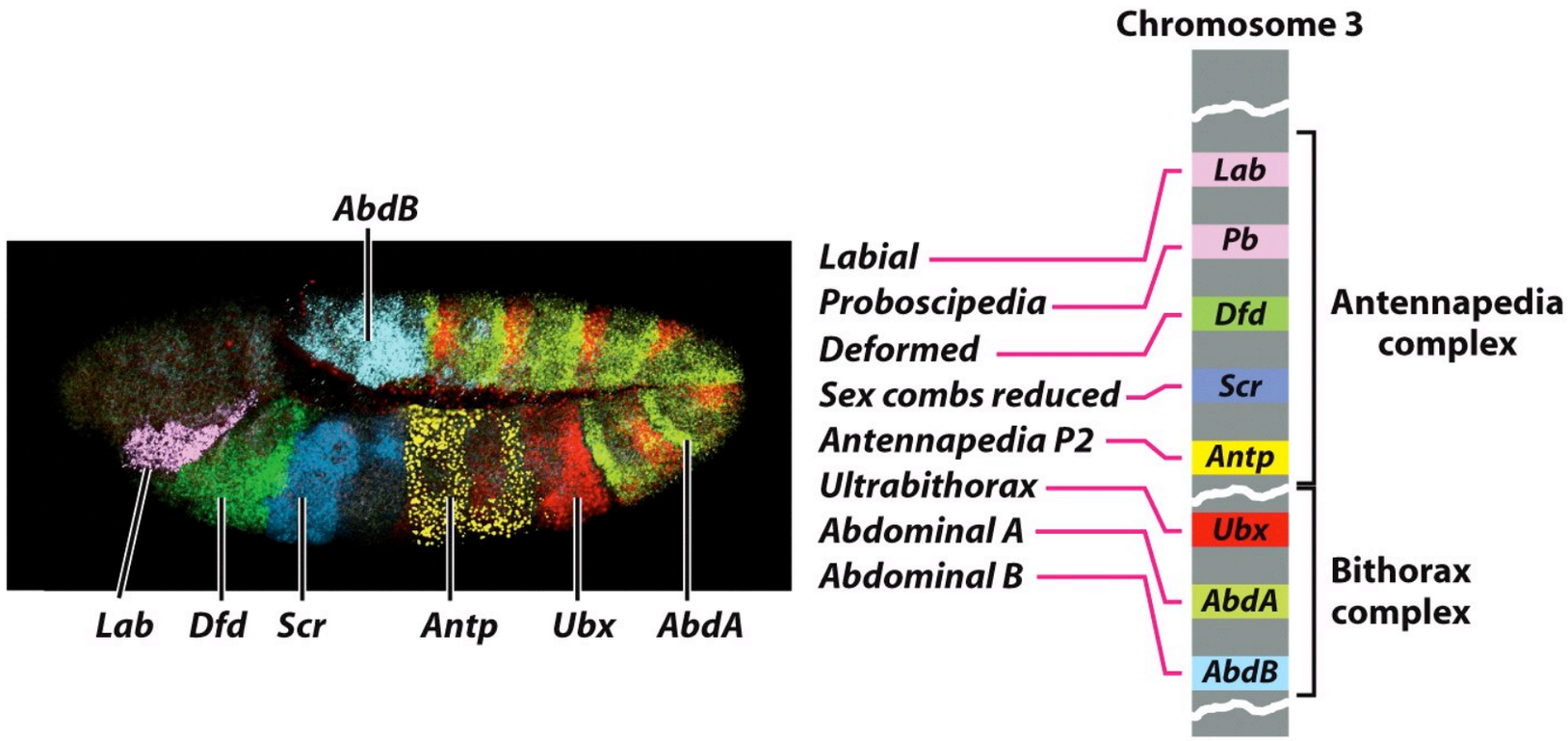


Figure 22-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

These proteins contain 60 amino acids of a conserved DNA-binding domain called the homeodomain.

These genes are located in two clusters (*Antennapedia* complex and *Bithorax* complex) on chromosome 3

The order of genes on the chromosome corresponds almost entirely to the order in which they are expressed along the AP axis of the body (**co-lineality**)

A-P axis in vertebrates is also controlled by Hox genes

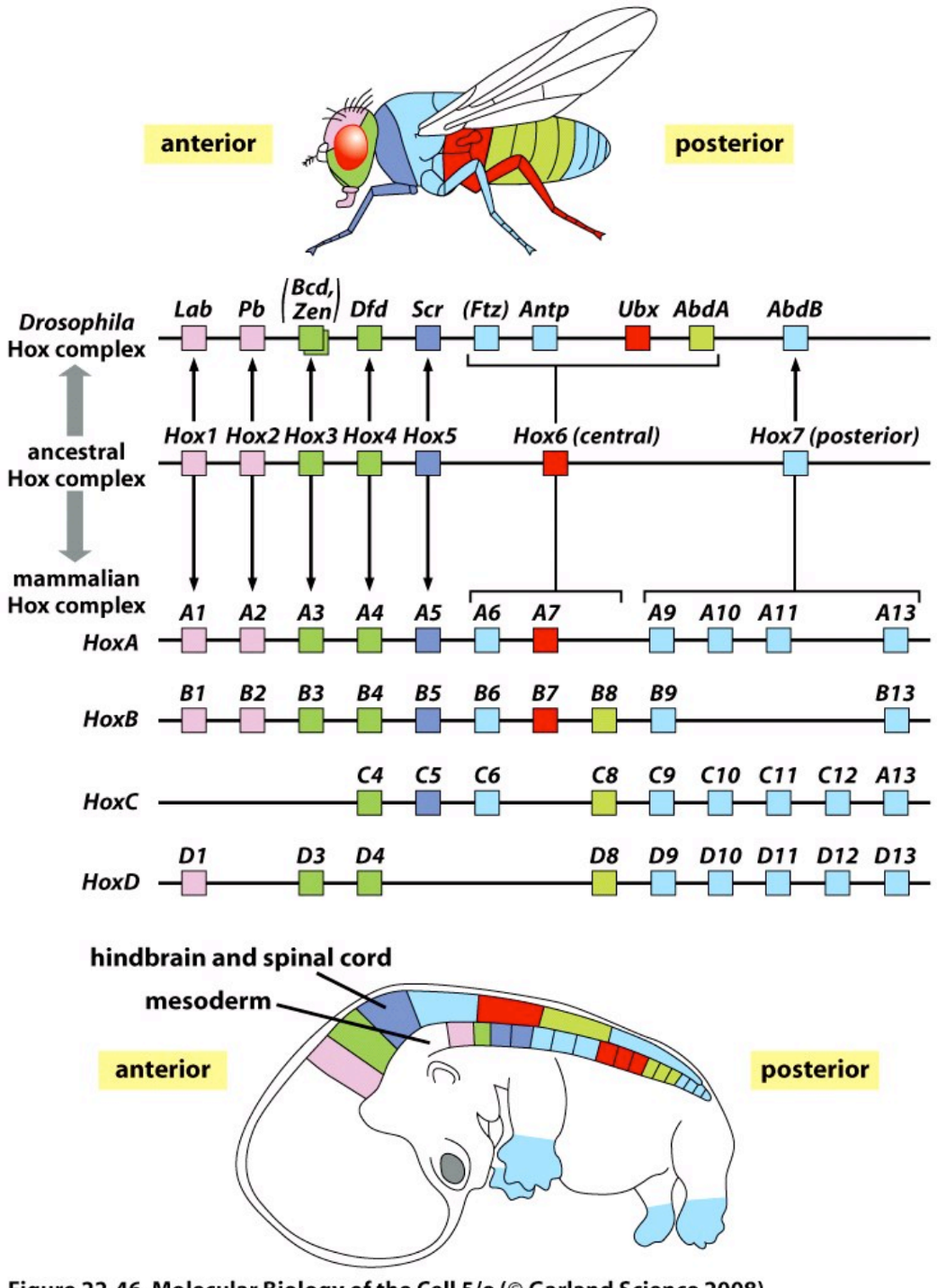


Figure 22-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

In the mouse, there are four complexes, *HoxA*, *HoxB*, *HoxC* and *HoxD* complexes, each on different chromosomes.

Each of the four complexes is the equivalent of the Drosophila set.

Members of each complex are expressed in a head-to-tail series along the AP axis, just as in Drosophila (the pattern is most clearly seen in the neural tube, from the hindbrain to the spinal cord, but is visible in other tissues such as the mesoderm).

Regulation and functions of the Hox genes in vertebrate nervous system will be discussed later.

Hox genes ≠ Homeobox genes

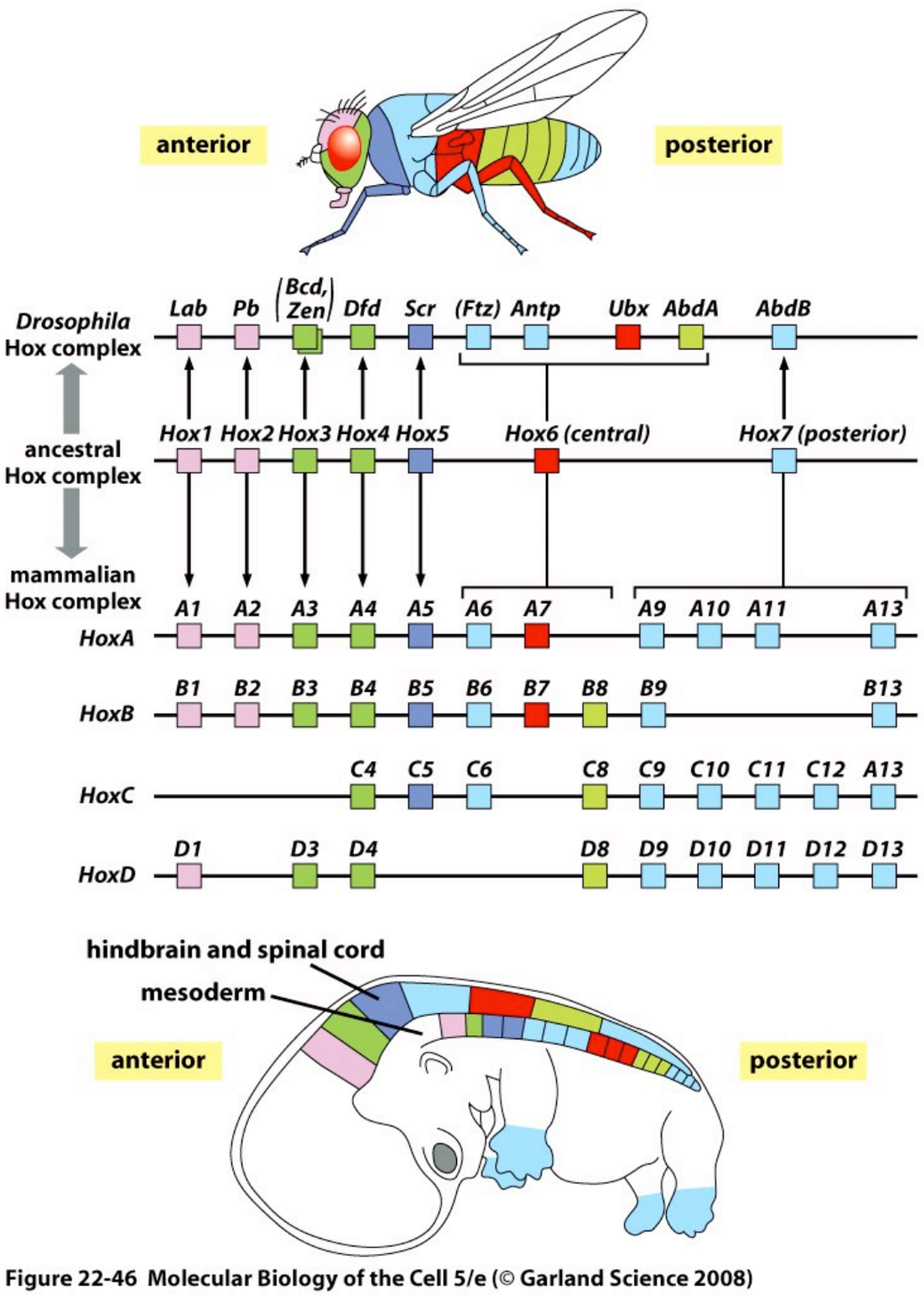


Figure 22-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Homeobox: 180 nucleotide DNA sequence (encoding 60 amino acid of the conserved DNA-binding domain called the *homeodomain*

Homeobox genes: genes containing a homeobox

Hox genes: genes on the Hox cluster on Drosophila chromosome or the Hox A-D clusters in the vertebrates (some vertebrates have fewer than four clusters). They only comprise a small portion of homeobox genes.

In the vertebrate brain, Hox genes are not expressed rostral to the hindbrain. Many homeobox genes that are not Hox genes are expressed in the midbrain and forebrain.

"Homeotic": functional term that describes the homeotic transformation (not the same as homeobox)

Summary of part 2

Signaling pathways and molecules involved in regionalization of the vertebrate nervous system were initially discovered in fly genetics

-Signaling pathway

- •cascade of gene regulation
  - egg polarity genes, gap genes, segment polarity genes, homeotic selector genes (transcription factors, signaling pathways of secreted molecules)
- •graded expression of transcription factors forms discrete boundaries within embryos

-Molecules

- •*Hox* genes (encodes a family of transcription factors) are involved in identity of specific body segments.
  - -*Hox* genes and other homeobox genes encode homeodomain-containing transcription factors.
  - -co-lineality (Hox genes are not expressed in rostral neural tissue)
  - -conserved in vertebrates
- •secreted signaling molecules and their receptors
  - Wingless (≃Wnt in vertebrates)
  - Hedgehog (≃Shh in vertebrates)