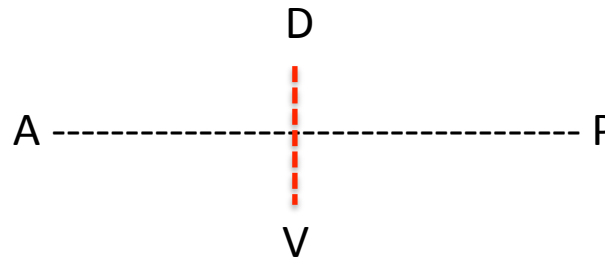


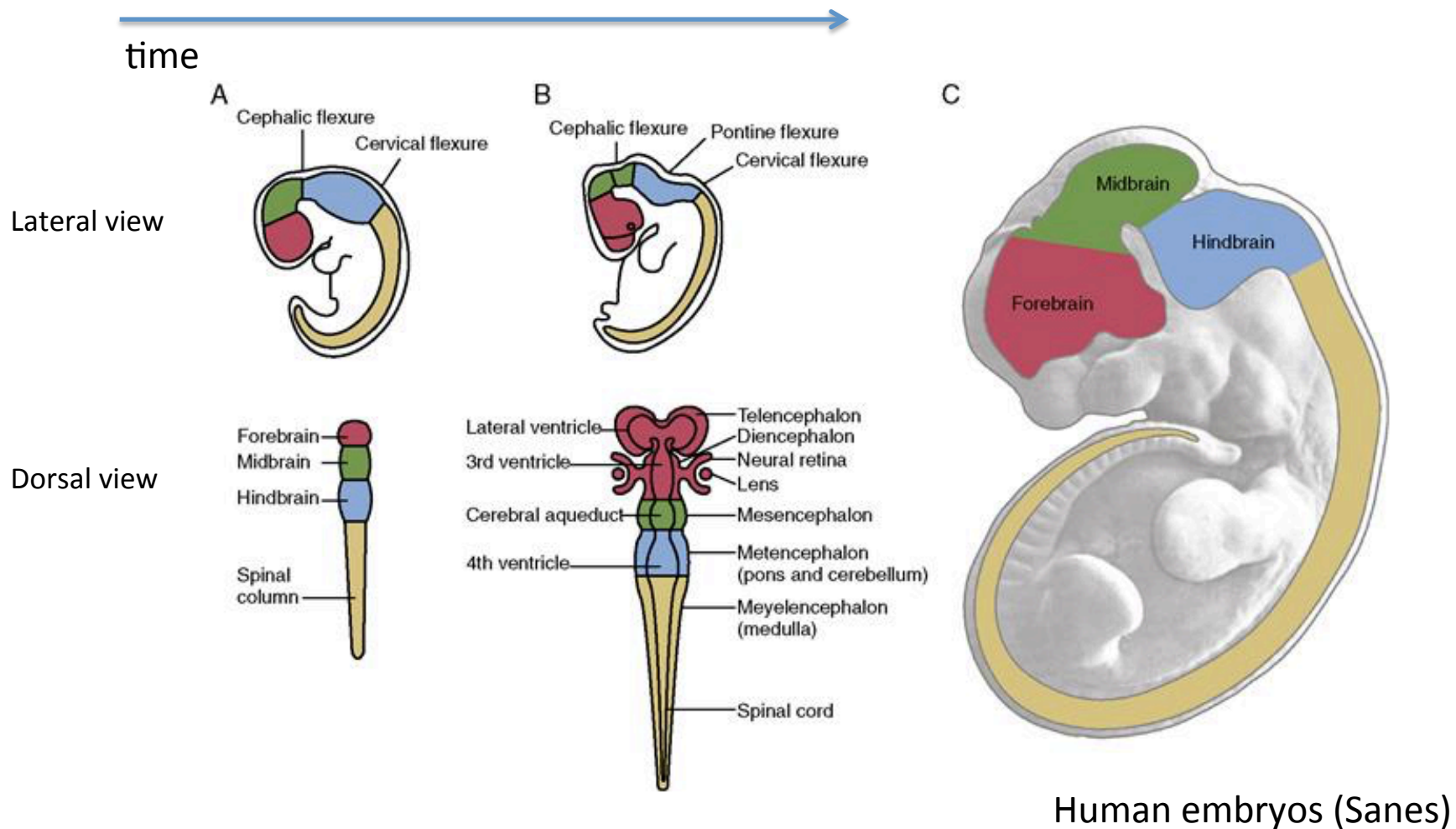
Patterning of the neural tube



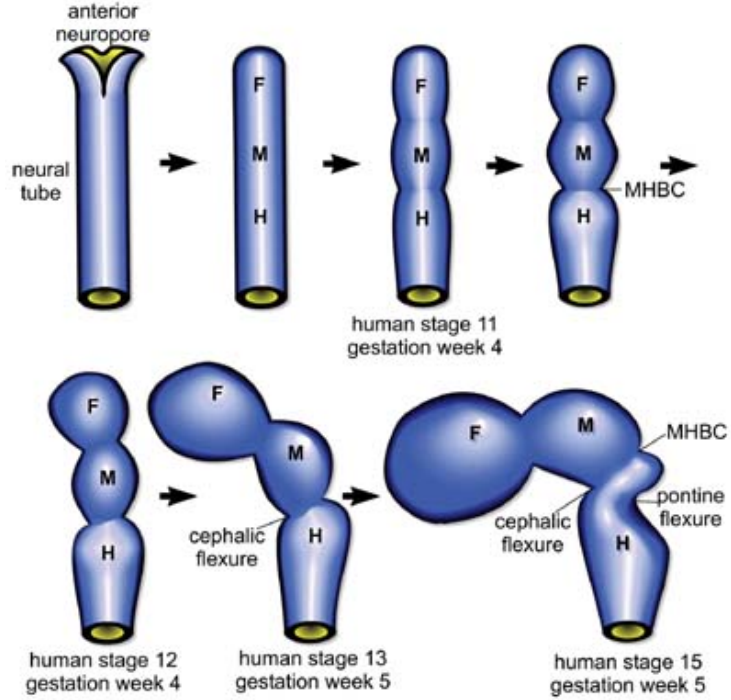
- Pattern formation is the process by which embryonic cells form ordered spatial arrangements within tissues.
- Within the neural tissue, the wide array of neurones and glial cells must be produced in a highly organized **temporal** and **spatial** order to form a functional nervous system

The nervous system is regionally specified during development

By the time the anterior neural tube has closed, the brain is clearly divided along the anterior–posterior (A–P) axis by a series of vesicles.



A Steps of Early Embryonic Brain Morphogenesis



human stage 11
gestation week 4

human stage 12
gestation week 4

human stage 13
gestation week 5

human stage 15
gestation week 5

Embryo
Crown Rump Length (CRL) 9 mm
Stage 15, 35 - 38 days, 7 - 9 mm



Neural Tube
Week 5



Embryo
Crown Rump Length (CRL) 11 mm
Stage 16, 37 - 42 days, 8 - 11 mm



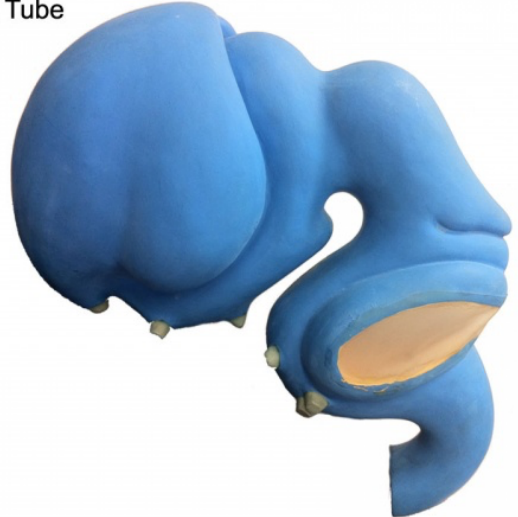
Neural Tube
Week 6



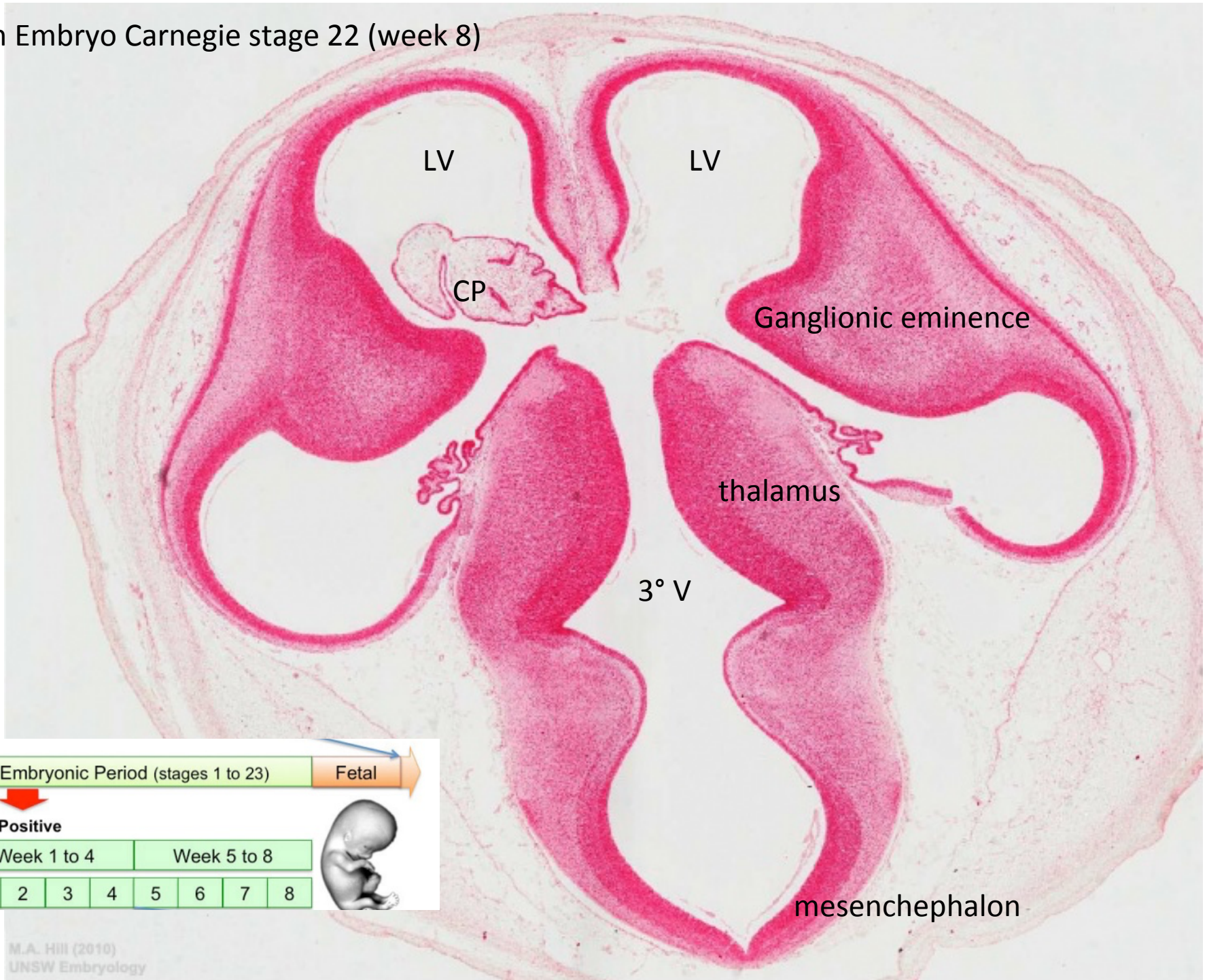
Embryo
Crown Rump Length (CRL) 27 mm
Stage 22, 54 - 56 days, 23 - 28 mm



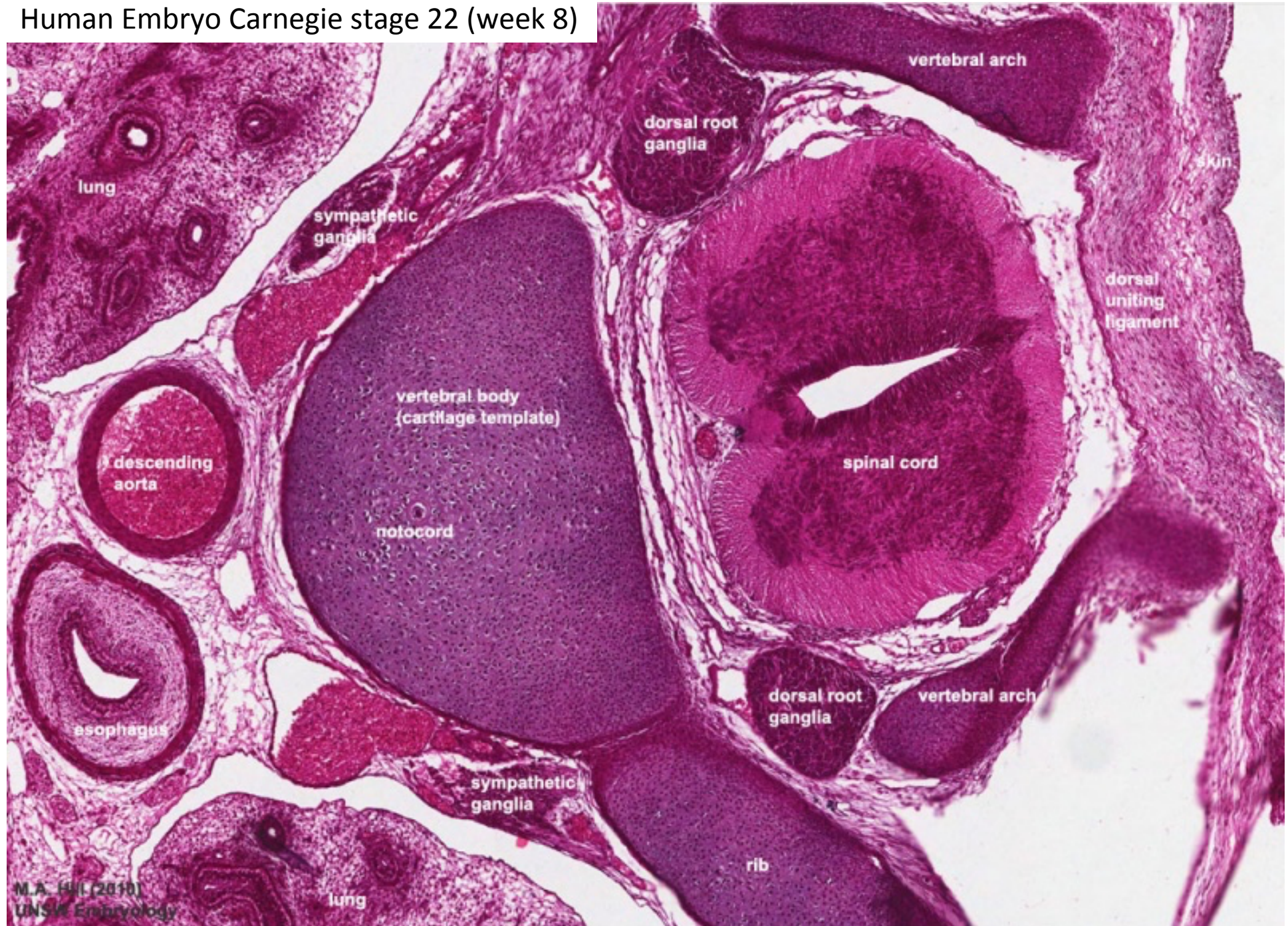
Neural Tube
Week 8



Human Embryo Carnegie stage 22 (week 8)

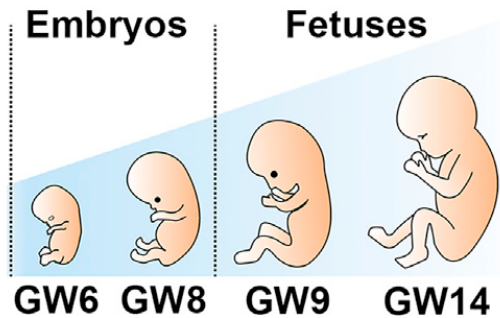


Human Embryo Carnegie stage 22 (week 8)



Tridimensional Visualization and Analysis of Early Human Development

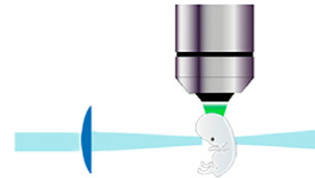
Collection of human embryonic and fetal specimens



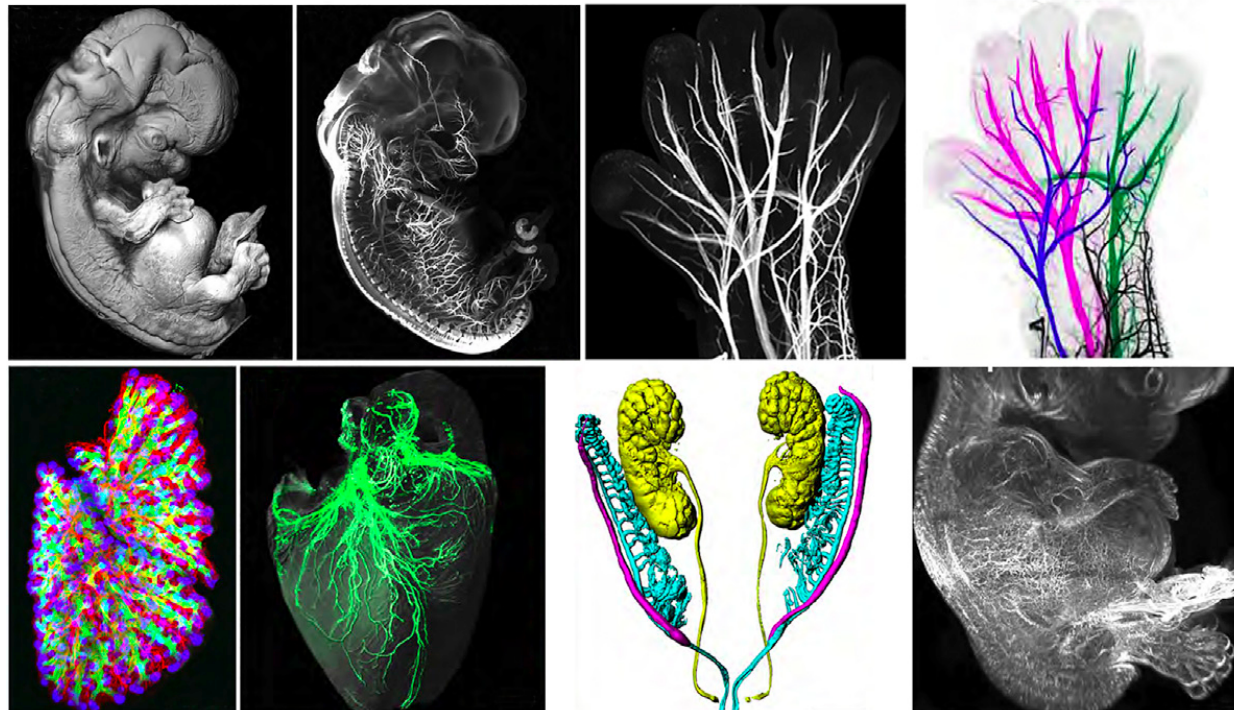
Whole-mount immunostaining
3DISCO clearing

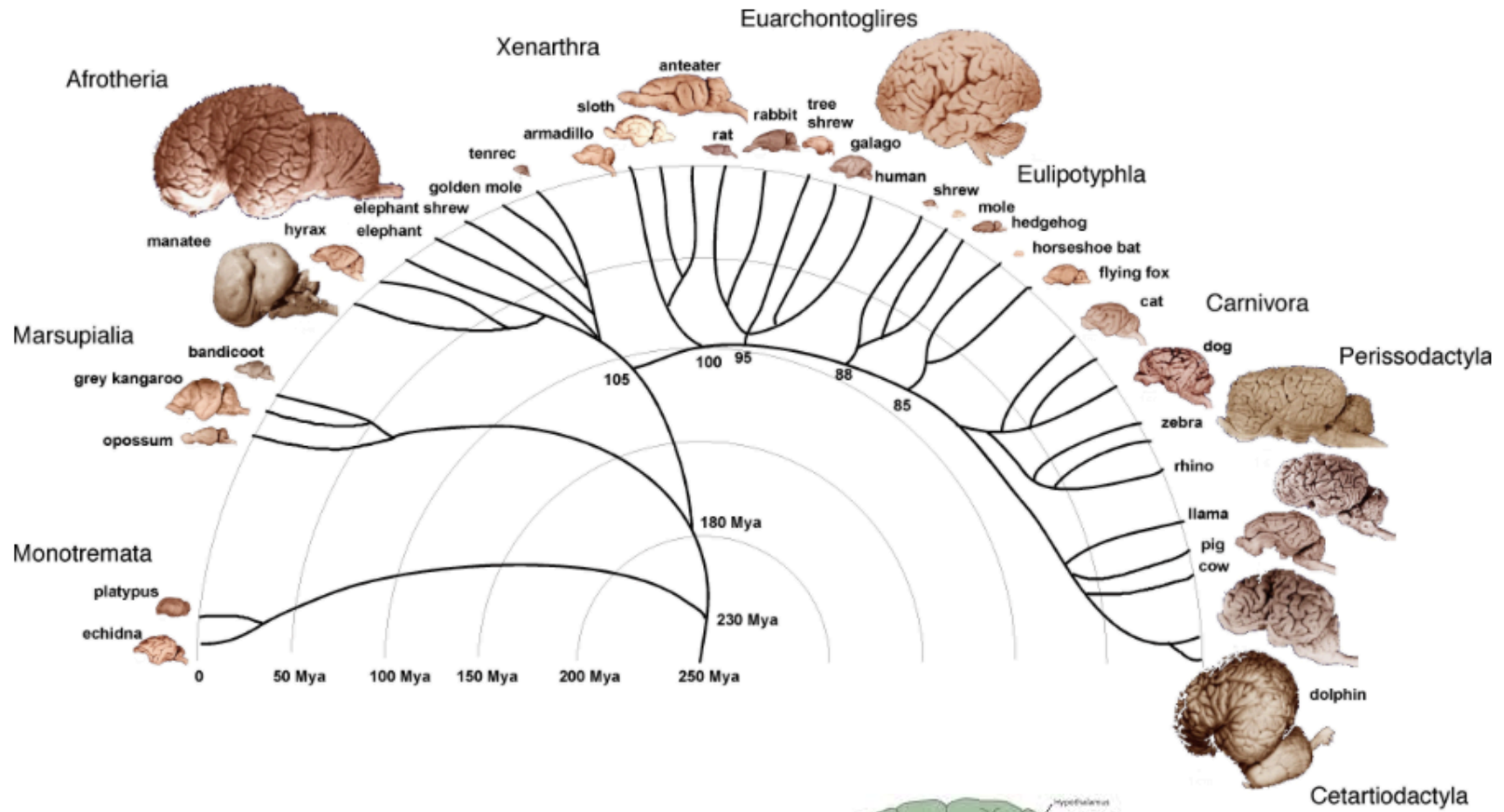


Light-sheet microscopy

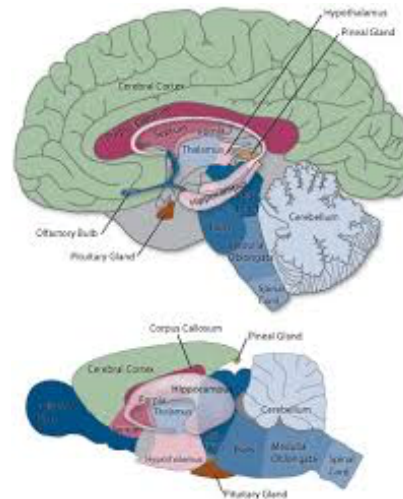
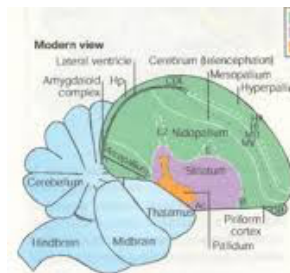
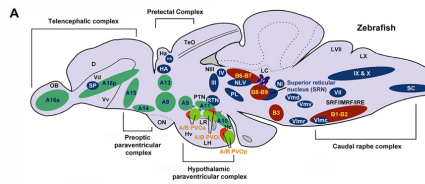


3D-Atlas of immunolabelled transparent human embryos/fetuses

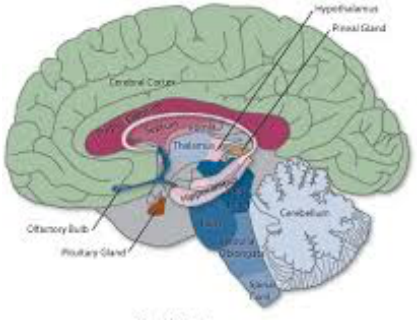
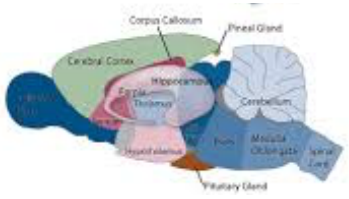
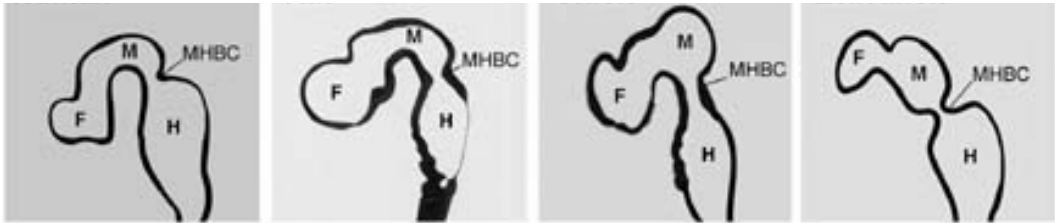
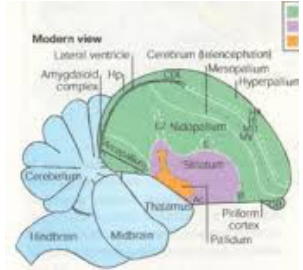
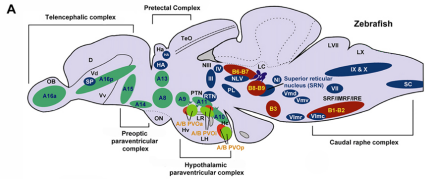




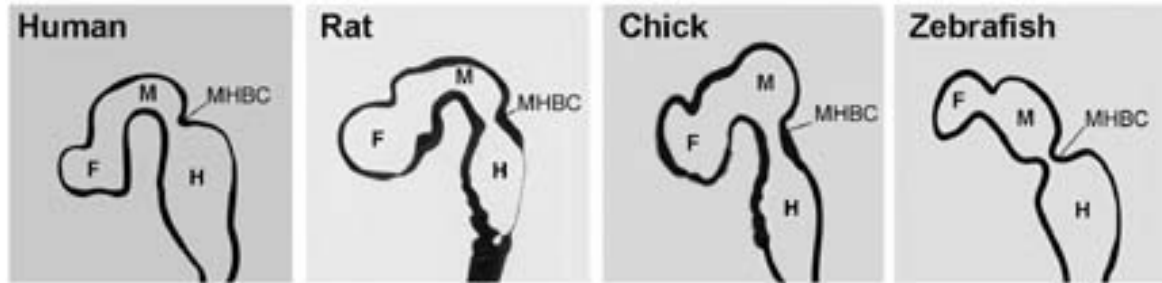
From Herculano-Houzel (2012) PNAS



The early development of most vertebrate brains is similar

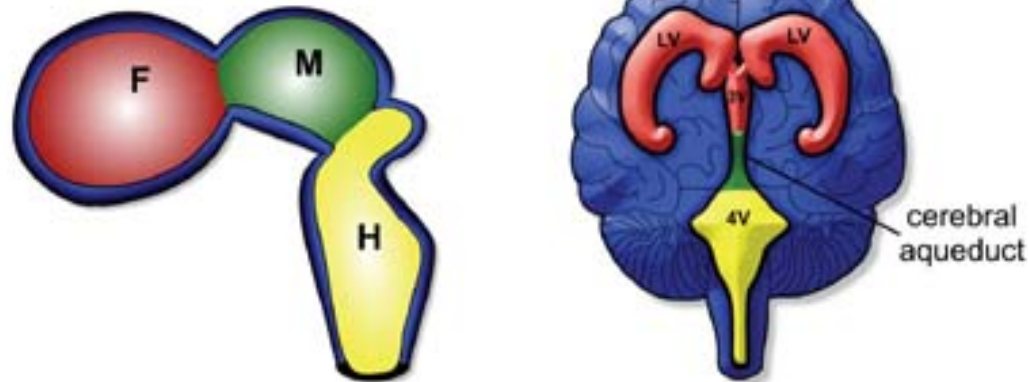


B Conservation of Embryonic Brain Ventricle Structure



Human: 43 dpf
Rat: 14 dpf
Chick: 2.5 dpf
Zebrafish: 24hpf

C Early Embryonic Brain Ventricles vs Adult Brain Ventricles



https://commons.wikimedia.org/wiki/File%3AHuman_Ventricular_system_colored_and_animated.gif

https://upload.wikimedia.org/wikipedia/commons/2/29/Hirnventrikel_mittelgro%C3%9F.gif

Comparative Neuroembryology

1920s Nils **Holmgren** comparative neuroembryological approach

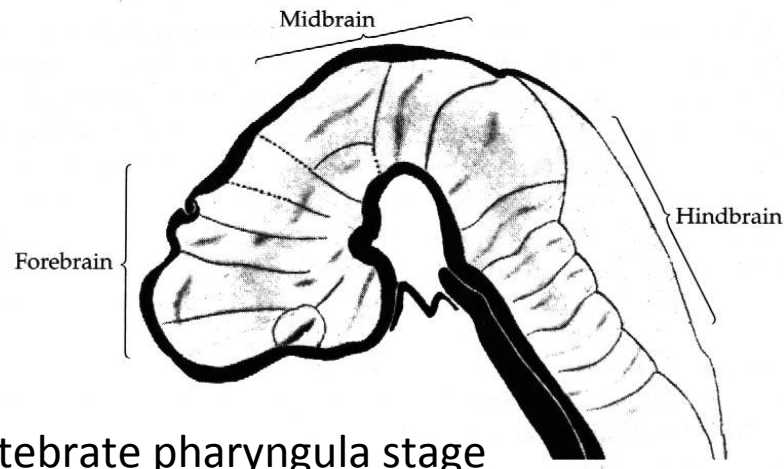
1953 Harry **Berquist** and Bengt **Kallén**

“All vertebrate embryonic brain shortly after neurulation exhibit a very similar pattern of organization “

Embryonic brain archetype (Bauplan)

Embryonic brains are divided into numerous transversely oriented proliferative zones (**neuromeres**) from which young neurons migrate toward their adult positions.

(A) Neuromeres according to Bergquist, 1952

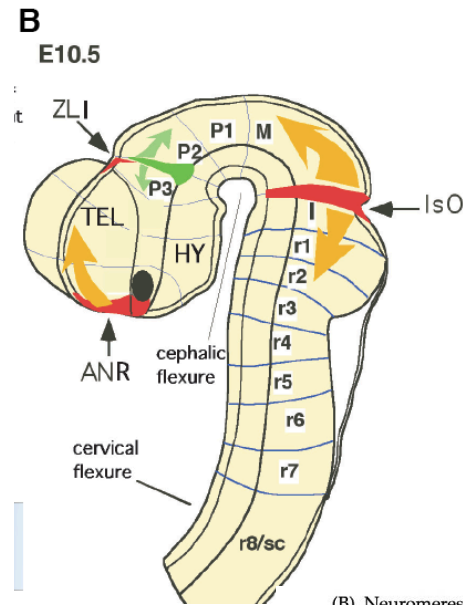


*By following the fate of these progenitors is possible to identify how developmental divergence creates adult species differences...and thus **clarify several homologies that were controversial based on adult forms...***

Vertebrate pharyngula stage

Neuromeres = series of **embryonic segmental units**, or compartments, from which differentiate the different parts of the vertebrate brain

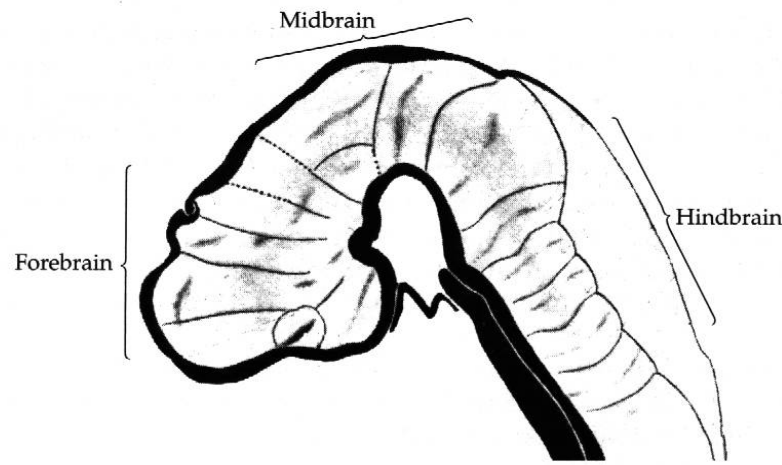
- Prosomeres
- Mesomeres
- Rhombomeres



Compartmentalization into **morphogenetic units**

positional identity is maintained by **CLONAL RESTRICTION**

(A) Neuromeres according to Bergquist, 1952



(B) Neuromeres according to Puelles et al., 1987

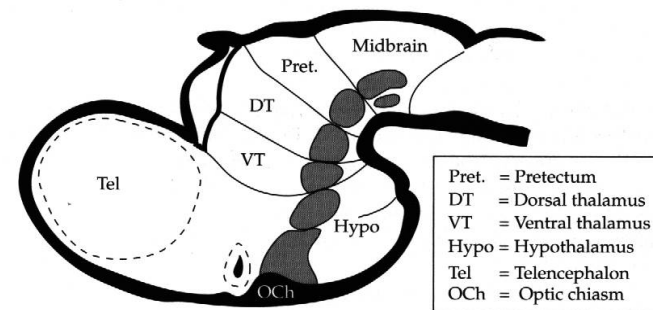


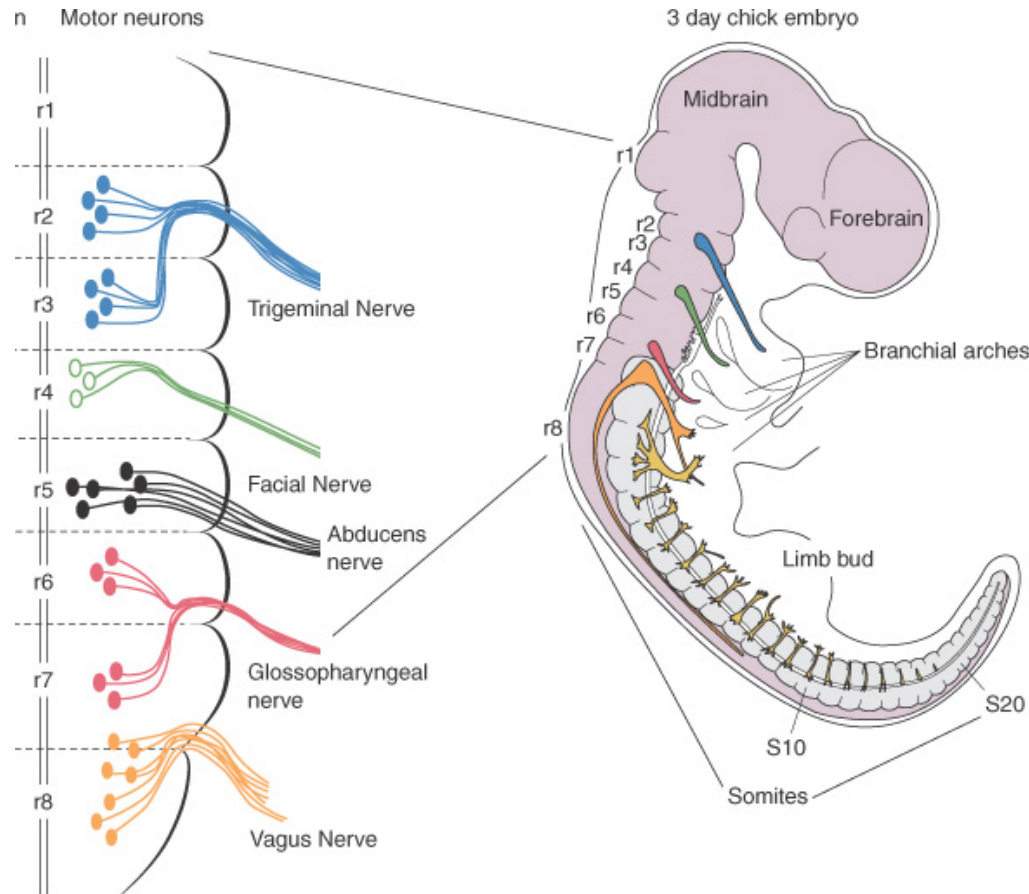
Figure 2.15 Versions of the "Neuromeric Model" Comparative neuroembryologists in Sweden during the first half of the twentieth century proposed that embryonic vertebrate brains are divisible into numerous transverse segments, or neuromeres, which are separated from one another by external grooves. This neuromeric model of vertebrate brain organization languished until it was revived by Luis Puelles, John Rubenstein, and others, who used histochemical and molecular biological methods to confirm that neuromeres exist. Shown in (A) is an illustration of the neuromeric model produced by Harry Bergquist in 1952; (B) is a diagram of AChE-positive cell clusters (gray) that Puelles et al. (1987) used to illustrate their version of the neuromeric model. (A after Bergquist, 1952; B after Puelles et al., 1987.)

Regionalization → generates diversity in cell types

Precursor cells become regionalized => their potential is based on their **spatial position**

- ✓ How does the CNS acquire regional specialization?
- ✓ How do the neurons (and glial cells) in the different regions of the brain and spinal cord become different?

Rhombomers in the developing hindbrain



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- Hindbrain rhombomeres are transient, serially homologous structures separated by distinct boundaries
- Each rhombomer gives rise to a unique set of motor neurons that control different muscle of the head



How do rhombomers become different to each other?

Hindbrain: region of the brain that coordinates motor activity, breathing rhythms, and many unconscious functions

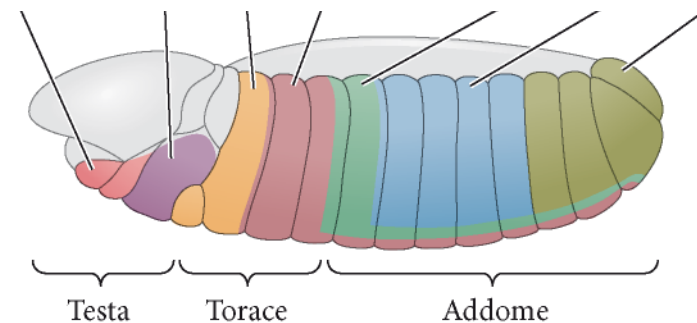
The regional development of the nervous system depends on mechanisms that initially set-up an antero-posterior pattern in the body: these factors have been initially studied and identified in drosophila

Antero-posterior axis in Drosophila

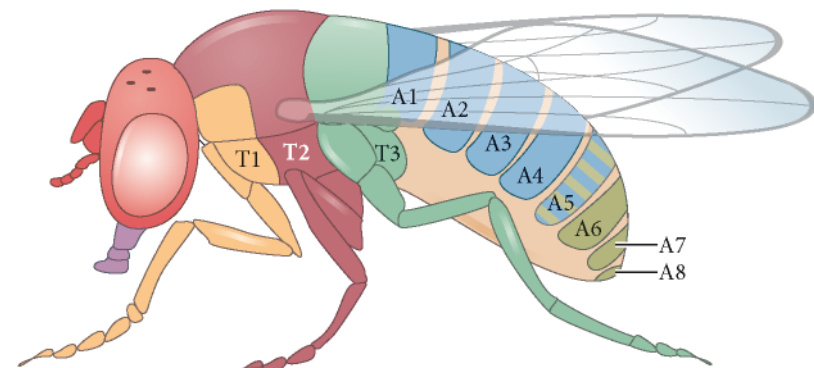
Each segment of the drosophila is unique

the unique properties of each segment depend upon POSITION along A-P axis

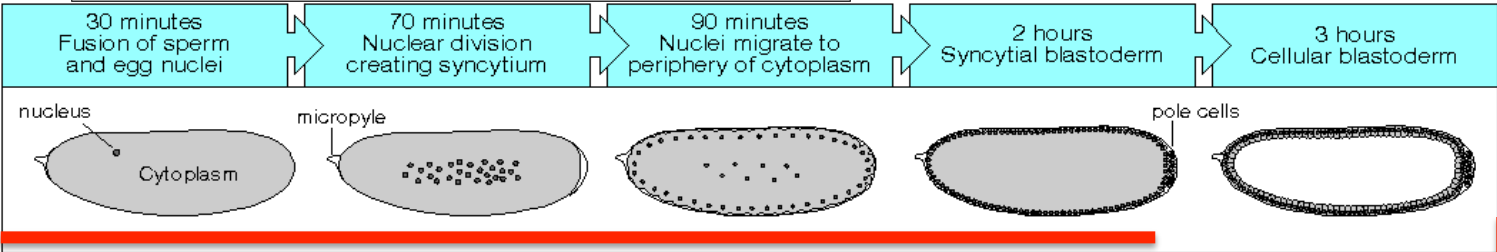
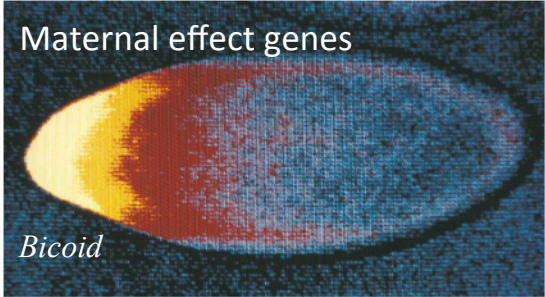
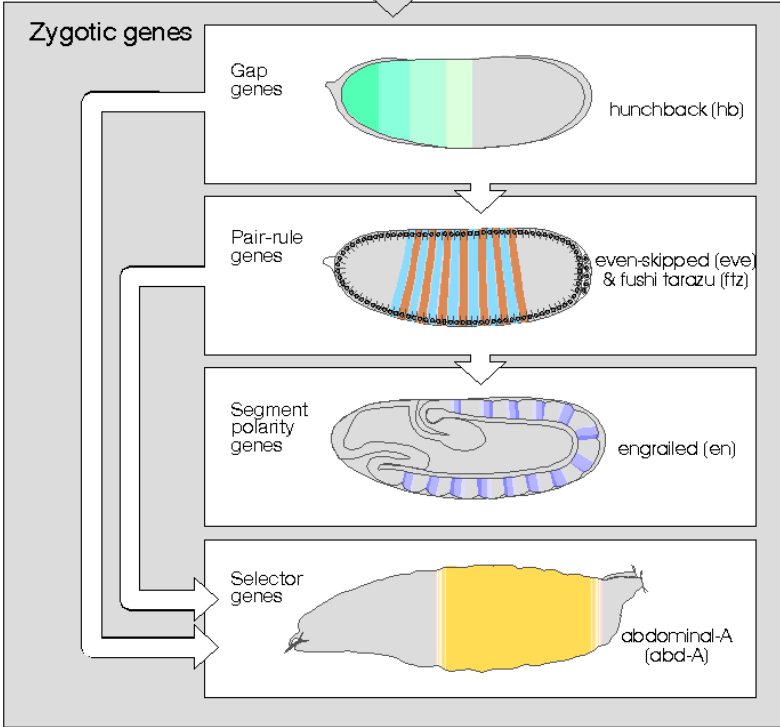
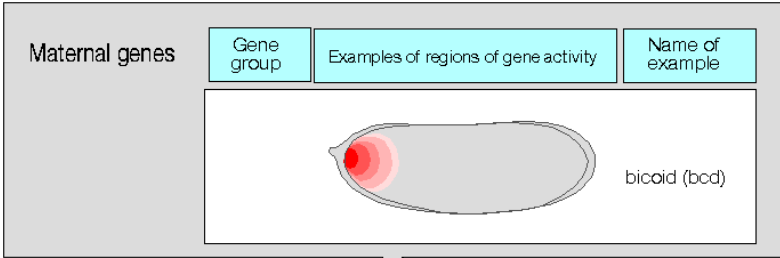
Appropriate specification of body axes during development requires the **precise control of a co-ordinated system of positional cues**



(A)



A: Bicoid
P: Nanos



Rapid cell cycle

Nuclear mitosis
No cytochinesis

Major onset of trascription

Zygotic genes

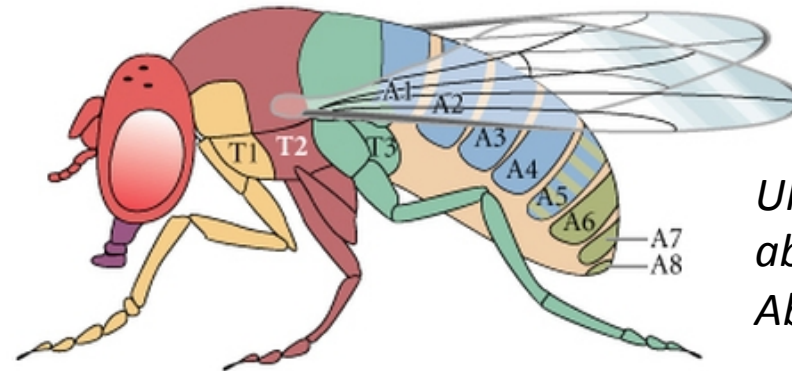
Distinct cell behaviours

gastrulation

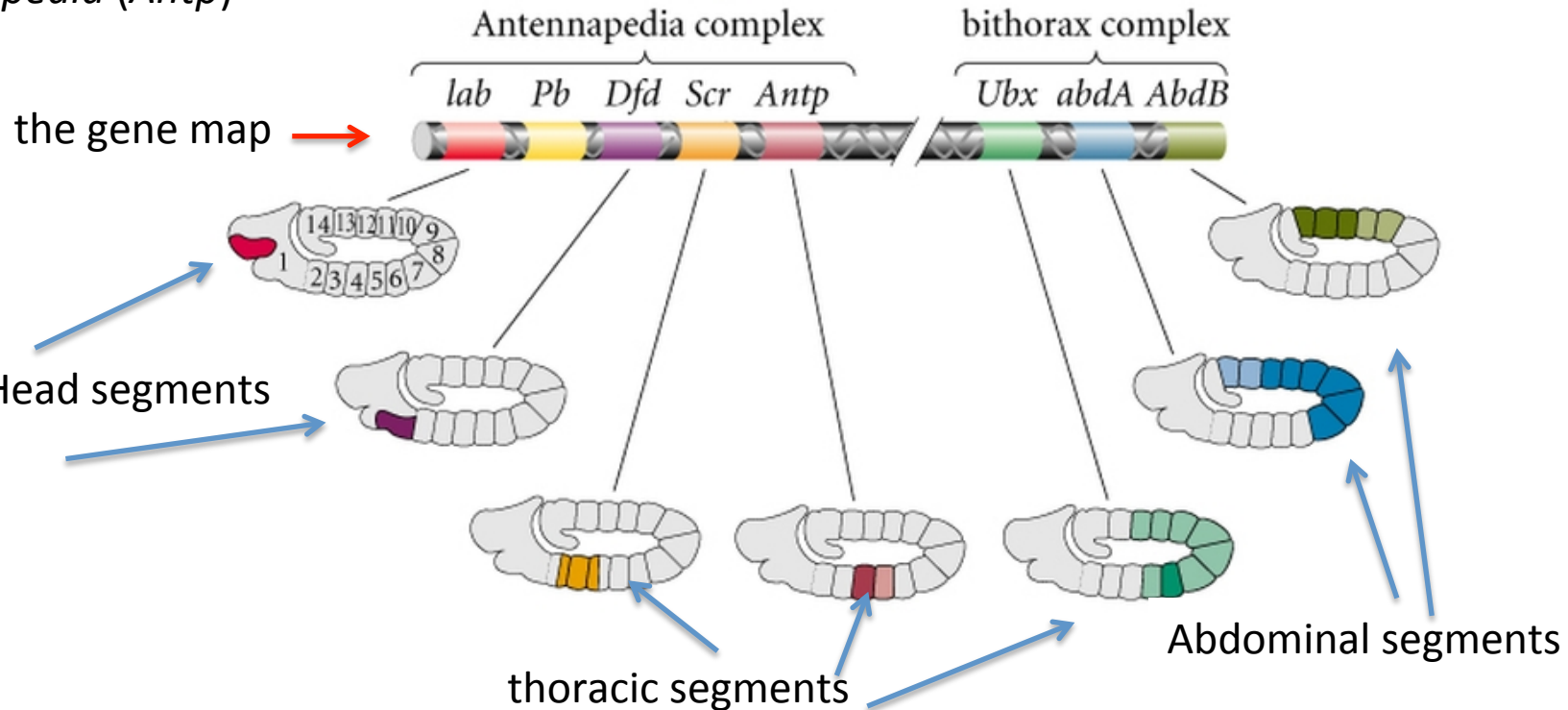
Drosophila chromosome 3 contains two clusters of the homeotic genes

The homeotic complex (Hom-C)

labial (lab)
proboscipedia (pb)
Deformed (dfd)
Sex combs reduced (scr)
Antennapedia (Antp)

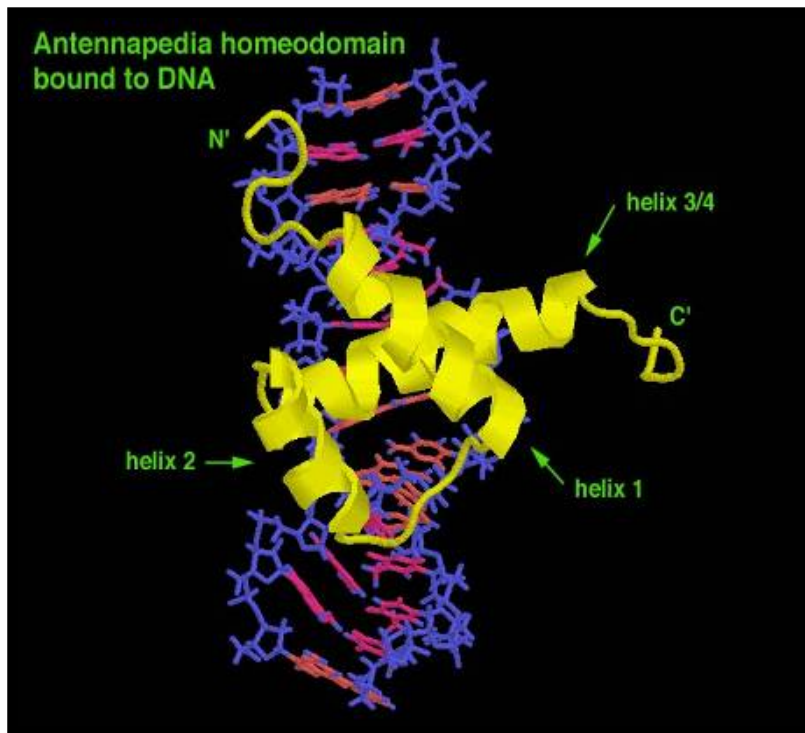


Ultrabithorax (ubx)
abdominal A (abdA)
Abdominal B (AbdB)



Homeotic genes

Each **Hom-C** gene contains a sequence of 180bp nucleotides known as a **homeobox**, which encodes a highly conserved 60 aa DNA binding domain named **homeodomain** (Transcription Factors)



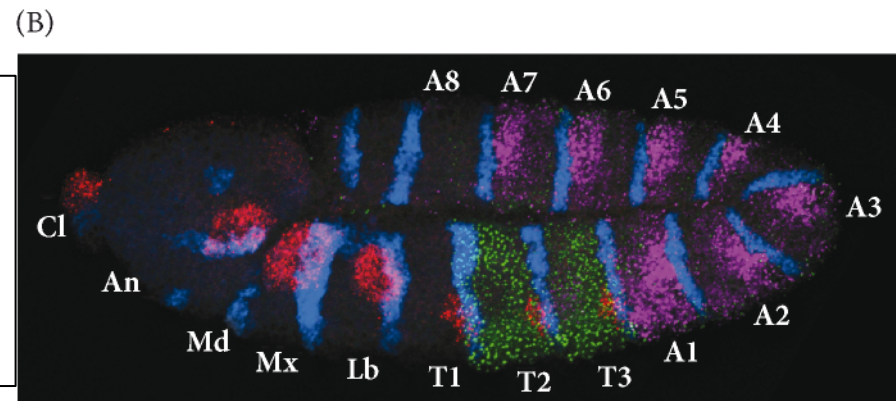
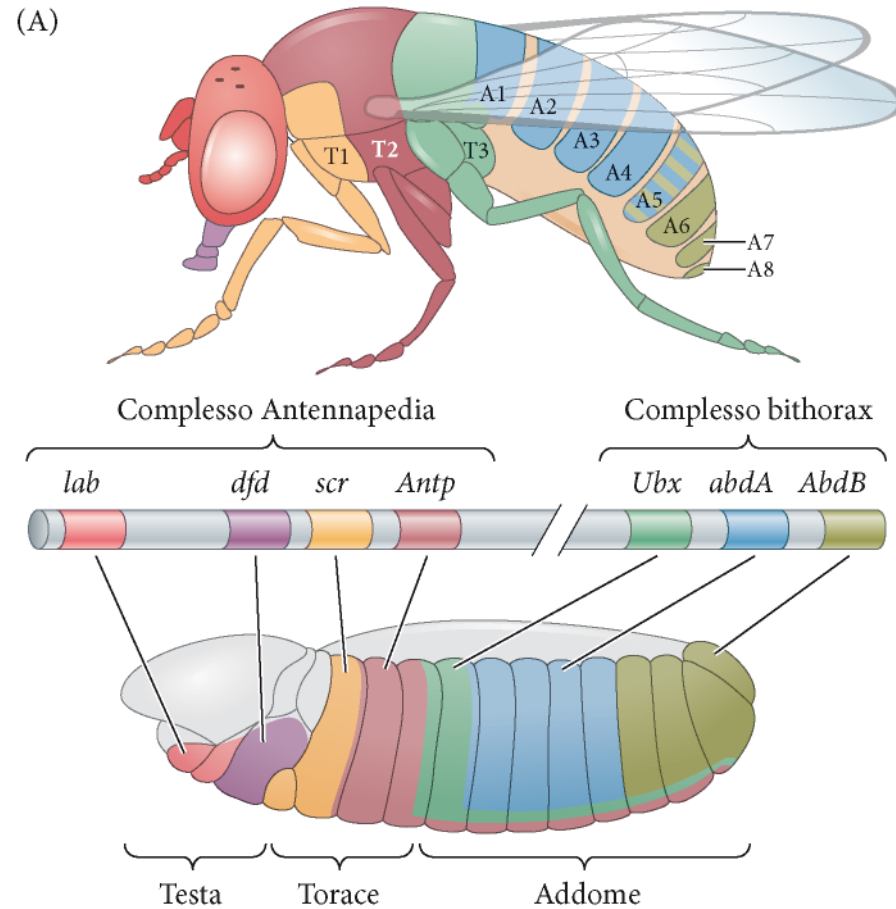
- A **homeotic protein** can **activate** one gene but **repress** another, producing effects that are complementary and necessary for the ordered development of an organism.
- A single homeotic protein can regulate transcription of hundreds of targets – interaction with other TFs enhances their specificity.

Lewis, E.B. (1978) A Gene Complex Controlling Segmentation in *Drosophila*. *Nature* 276, 565-570 - Nobel Prize in 1995

Co-linearity principle:

the organization of the homeotic genes on the chromosome is the same as the order of their expression along the anterior-posterior axis of the developing animal

genes which are located more 3' in the cluster are generally expressed at a more anterior position in the embryo [**spatial colinearity**] and also at an earlier developmental time point [**temporal colinearity**]



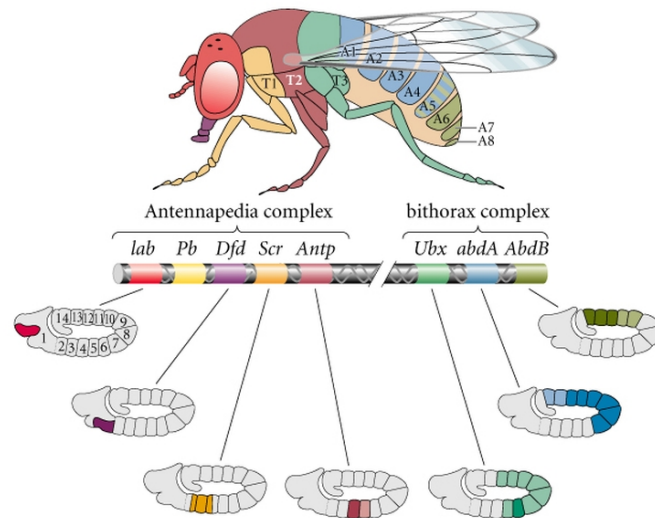
ISH
 Engrailed (blue)
 Antennapedia (green)
 Ultrabitorax (violet)
 Distal-less (red)

Maintaining the patterns of homeotic gene expression

The expression of **homeotic genes** is a dynamic process

Homeodomain TFs regulate each other's gene through complex regulatory networks

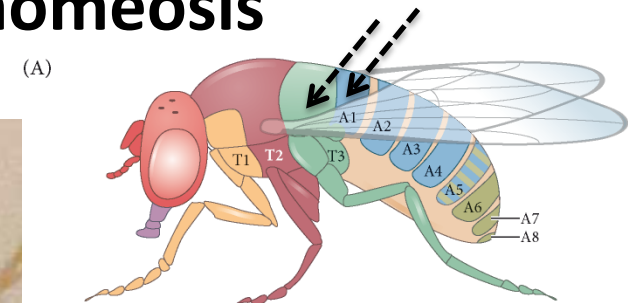
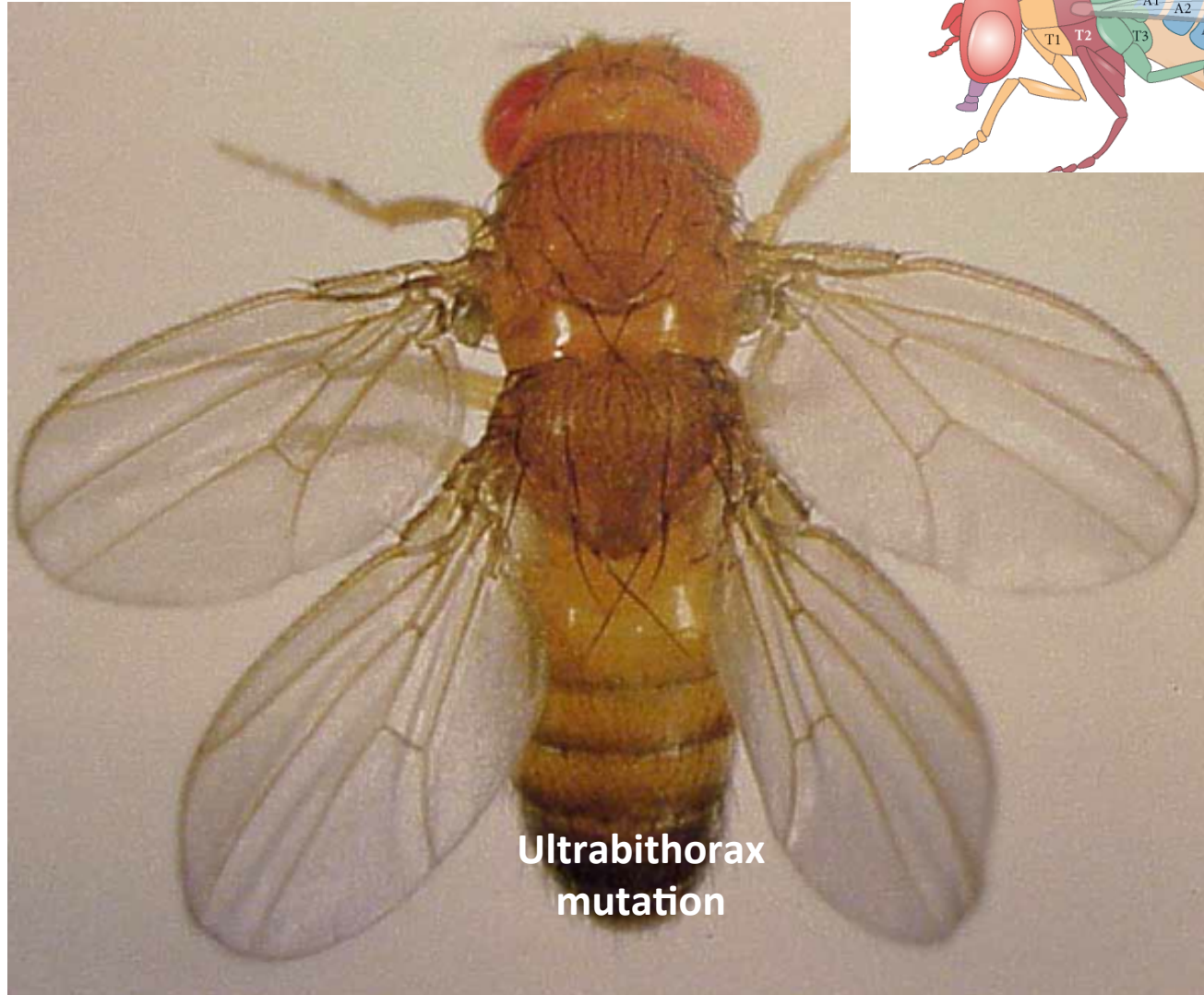
Posterior dominance: the activity of posterior Homeotic genes dominates over more anterior ones.



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- each of the bithorax complex **genes** represses the expression of *Antennapedia*
- If the *Ultrabithorax* gene is deleted, *Antp* activity extends through the region that would normally have expressed *Ubx*.

Homeotic genes are responsible for the specification of fly body parts, mutations in homeotic genes lead to **homeosis**



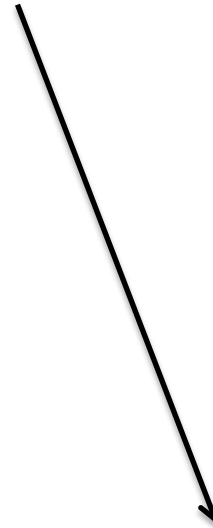
Homeotic genes

(master genes - TFs)

downstream target genes

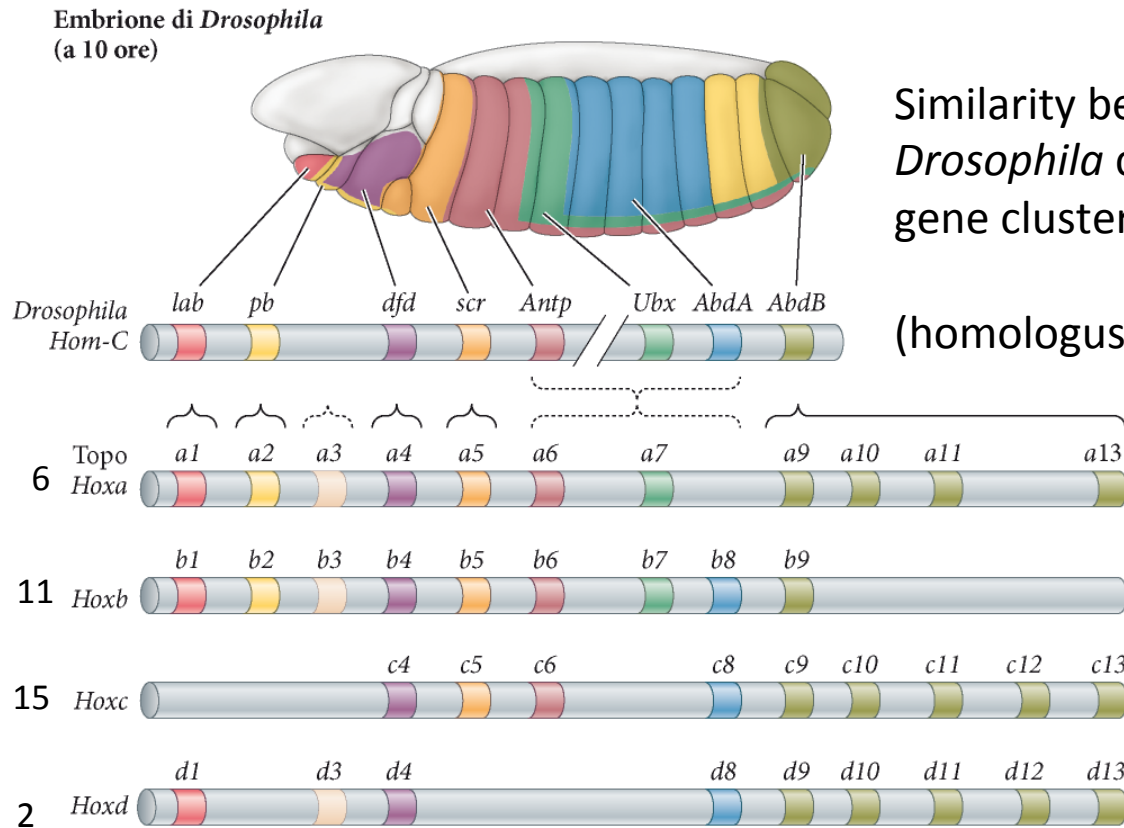


regulate **“regulator”** genes
that in turn control
large **networks** of other genes



directly regulate **effector (realizator) genes**
that act at the bottom of such hierarchies
to ultimately form the tissues, structures, and organs

Evolutionary conservation of homeotic gene organization and transcriptional expression in **fruit flies** and **mice**

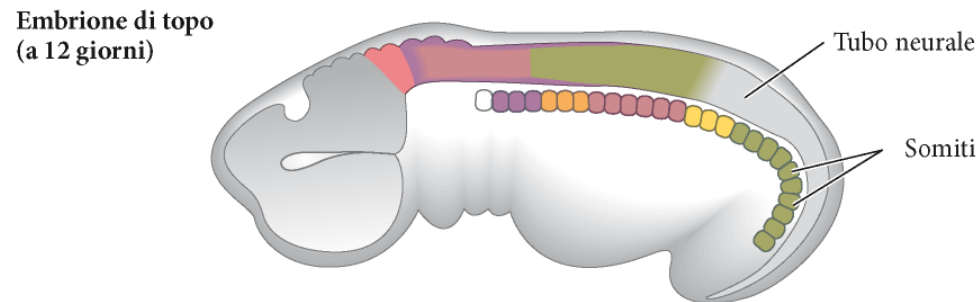


Similarity between the *Hom-C* cluster on *Drosophila* chromosome 3 and the four **Hox** gene clusters in the **mouse** genome

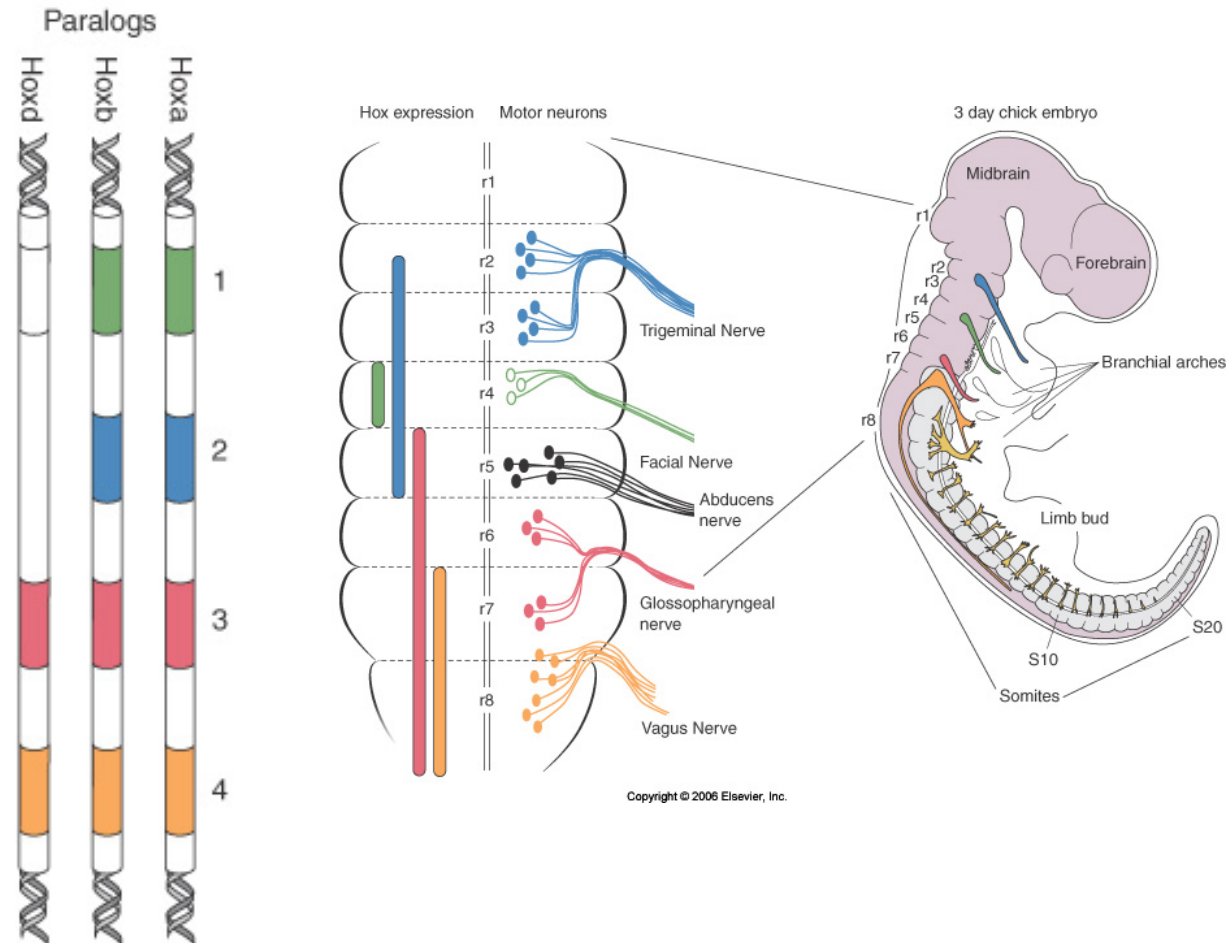
(homologous genes)

the order of the genes on the chromosomes has been conserved!

In the mouse, the Hox complex is made up of 39 genes distributed in paralogous clusters on four chromosomes (Hox A-D)

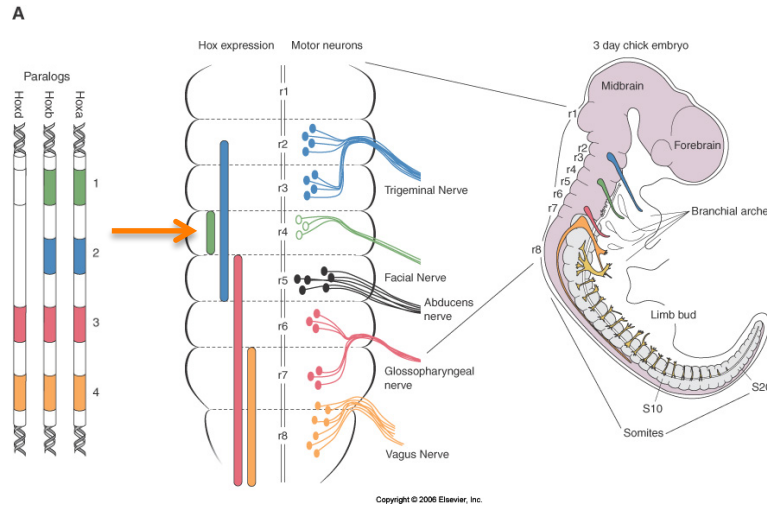


Hox genes function in vertebrate nervous system controlling the **regional identity** of the hindbrain



Combinatorial expression pattern code: members of *Hox* paralog groups 1–4 are expressed in overlapping rhombomere-restricted domains with the most anterior *Hox* gene, *Hoxa2*, being expressed up to the r1/r2 boundary → **pattern that is conserved among different vertebrates**

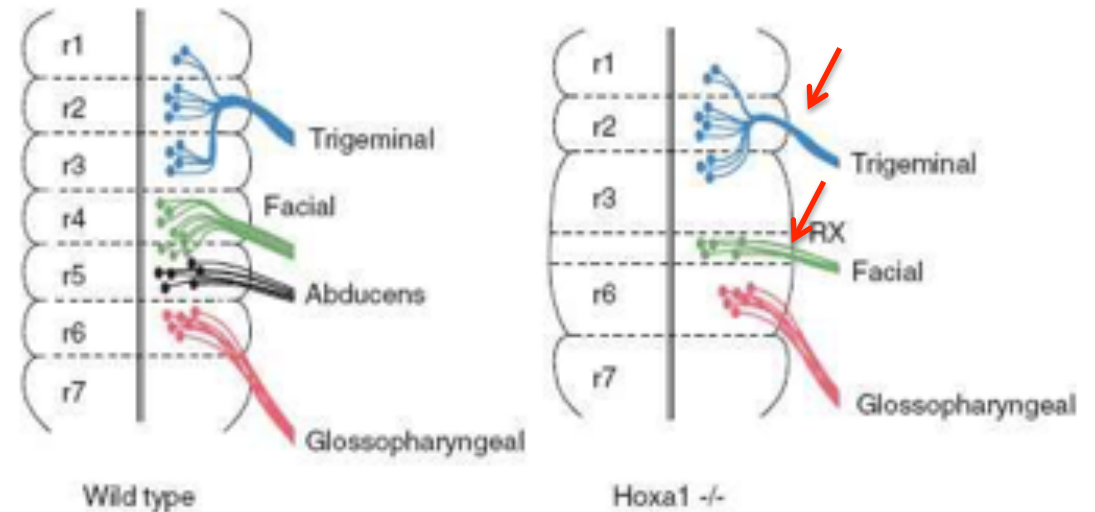
Loss- and gain-of-function analyses have shown that Hox genes control the specification of rhombomere identities in vertebrates



→ In mouse *Hox* knockouts, individual rhombomeres are frequently lost or partially transformed to more anterior identities

Hoxa1 $-/-$ → most severe phenotype

Defects in hindbrain and craniofacial development



Loss of *Hox-A1* (*Hox-1.6*) function results in the reorganization of the murine hindbrain

Ellen M. Carpenter, Judy M. Goddard, Osamu Chisaka, Nancy R. Manley and Marlo R. **Capecchi***

Howard Hughes Medical Institute, Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah 84112, USA

*Author for correspondence

SUMMARY

Targeted disruption of the murine *hox-A1* gene results in severe defects in the formation of the hindbrain and associated cranial ganglia and nerves. Carbocyanine dye injections were used to trace afferent and efferent projections to and from the hindbrain in *hox-A1*⁻/*hox-A1*⁻ mutant mice. Defects were observed in the position of efferent neurons in the hindbrain and in their projection patterns. In situ hybridization was used to analyze the transcription pattern of genes expressed within specific rhombomeres. *Krox-20*, *int-2* (*fgf-3*), and *hox-B1* all display aberrant patterns of expression in *hox-A1*⁻ mutant embryos. The observed morphological and

molecular defects suggest that there are changes in the formation of the hindbrain extending from rhombomere 3 through rhombomere 8 including the absence of rhombomere 5. Also, motor neurons identified by their axon projection patterns which would normally be present in the missing rhombomere appear to be respecified to or migrate into adjacent rhombomeres, suggesting a role for *hox-A1* in the specification of cell identity and/or cell migration in the hindbrain.

Key words: *Hox* genes, rhombomere, segmentation, mouse development

Knockdown of the complete Hox paralogous group 1 leads to dramatic hindbrain and neural crest defects

Claire L. McNulty*, João N. Peres*, Nabila Bardine, Willem M. R. van den Akker and Antony J. Durston†

Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

*These authors contributed equally to this work

†Author for correspondence (e-mail: tony@niob.knaw.nl)

Summary

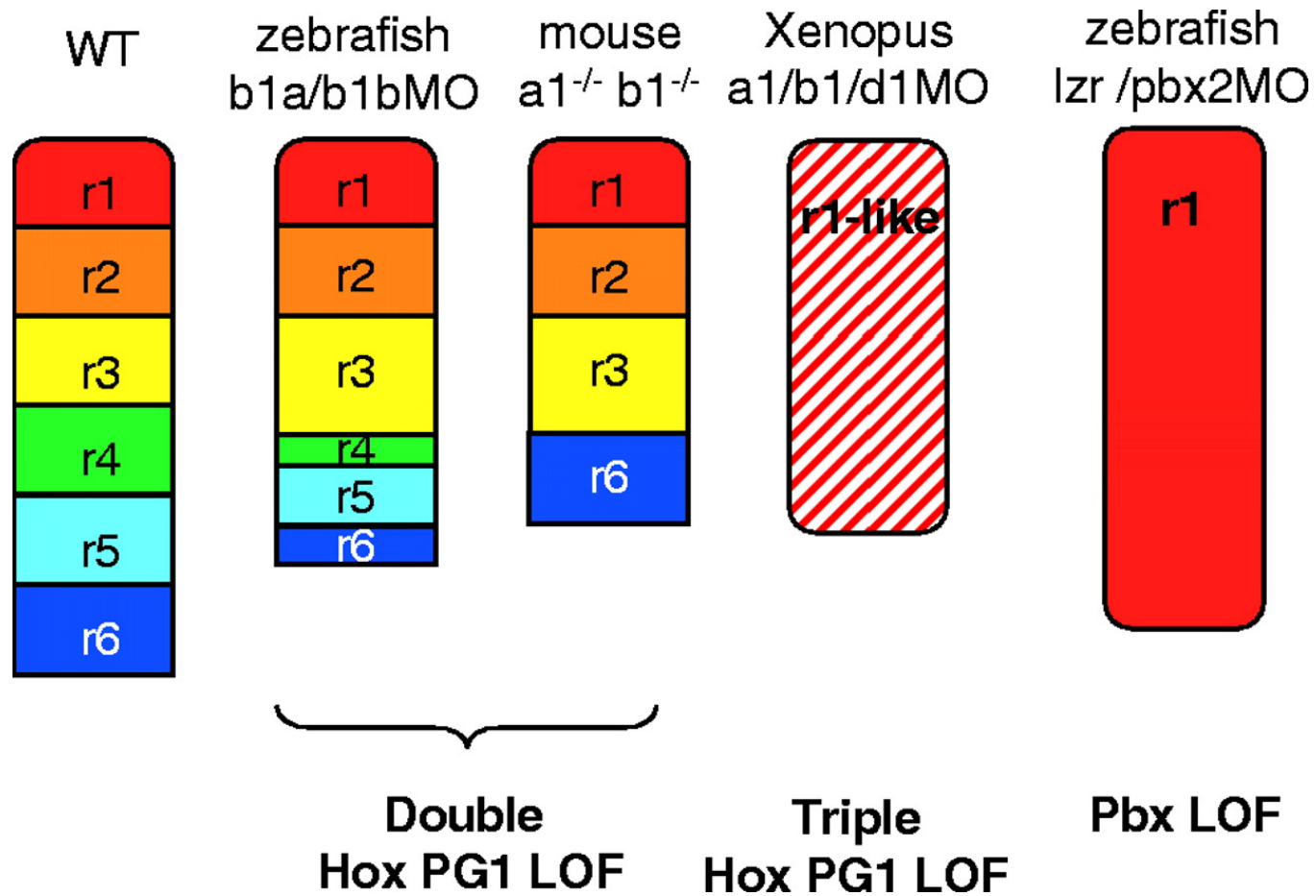
The Hox paralogous group 1 (PG1) genes are the first and initially most anterior Hox genes expressed in the embryo.

In *Xenopus*, the three PG1 genes, *Hoxa1*, *Hoxb1* and *Hoxd1*, are expressed in a widely overlapping domain, which includes the region of the future hindbrain and its associated neural crest. We used morpholinos to achieve a complete knockdown of PG1 function. When *Hoxa1*, *Hoxb1* and *Hoxd1* are knocked down in combination, the hindbrain patterning phenotype is more severe than in the single or double knockdowns, indicating a degree of redundancy for these genes. In the triple PG1 knockdown embryos the hindbrain is reduced and lacks segmentation. The patterning of rhombomeres 2 to 7 is lost, with a concurrent posterior expansion of the rhombomere 1

marker, *Gbx2*. This effect could be via the downregulation of other Hox genes, as we show that PG1 function is necessary for the hindbrain expression of Hox genes from paralogous groups 2 to 4. Furthermore, in the absence of PG1 function, the cranial neural crest is correctly specified but does not migrate into the pharyngeal arches. Embryos with no active PG1 genes have defects in derivatives of the pharyngeal arches and, most strikingly, the gill cartilages are completely missing. These results show that the complete abrogation of PG1 function in *Xenopus* has a much wider scope of effect than would be predicted from the single and double PG1 knockouts in other organisms.

Key words: Hox, PG1, *Xenopus*, Hindbrain, Neural crest

→the complete loss of PG1 gene function has deeper implications for the development of the embryo than the loss of the individual genes (redundancy)



Hox proteins cooperate with the **Pbx** and **Meis** homeodomain proteins to achieve their **DNA binding specificity**

Eliminating zebrafish pbx proteins reveals a hindbrain ground state.

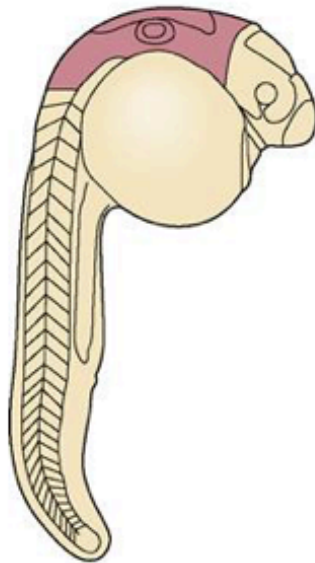
Waskiewicz AJ¹, Rikhof HA, Moens CB.

⊕ Author information

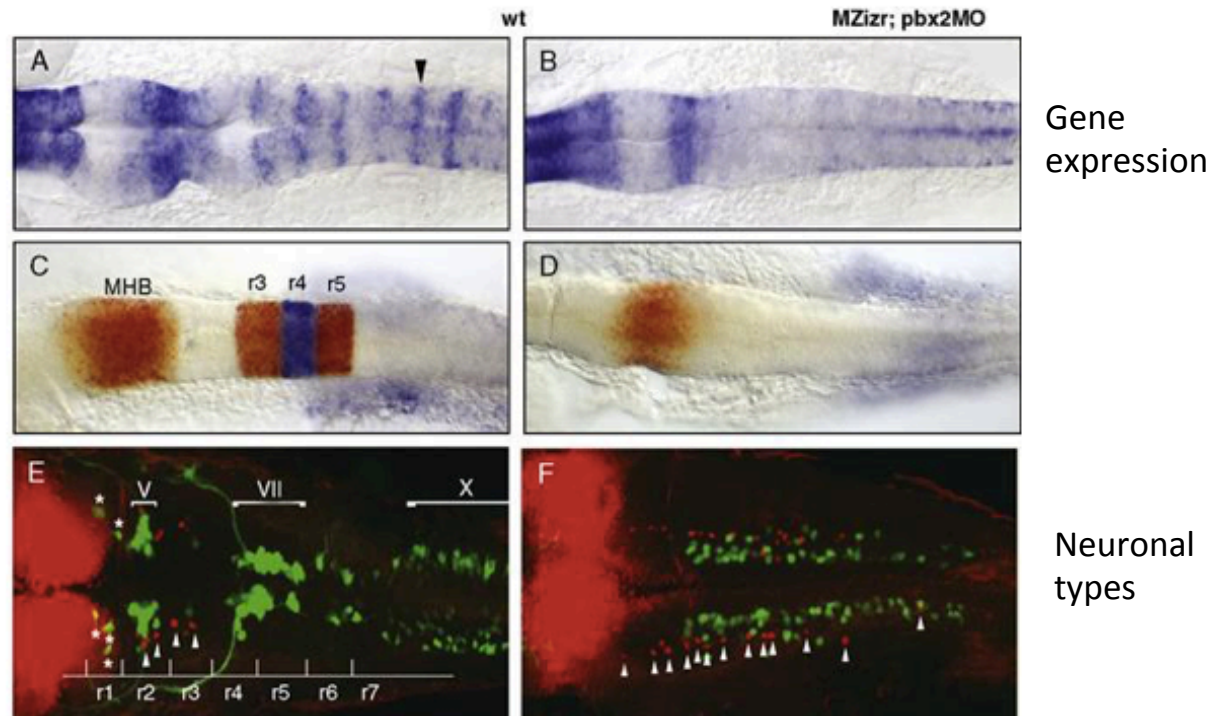
Abstract

The vertebrate hindbrain is divided into serially homologous segments, the rhombomeres (r). Pbx and Hox proteins are hypothesized to form heterodimeric, DNA binding transcription complexes which specify rhombomere identities. Here, we show that eliminating zebrafish Lzr/Pbx4 and Pbx2 function prevents hindbrain segmentation and causes a wholesale anterior homeotic transformation of r2-r6. to r1 identity. We demonstrate that Pbx proteins interact with Hox paralog group 1 proteins to specify segment identities broadly within the hindbrain, and that this process involves the Pbx:Hox-1-dependent induction of Fgf signals in r4. We propose that in the absence of Pbx function, r2-r6 acquire a homogeneous ground state identity, that of r1, and that Pbx proteins, functioning primarily with their Hox partners, function to modify this ground state identity during normal hindbrain development.

Pbx

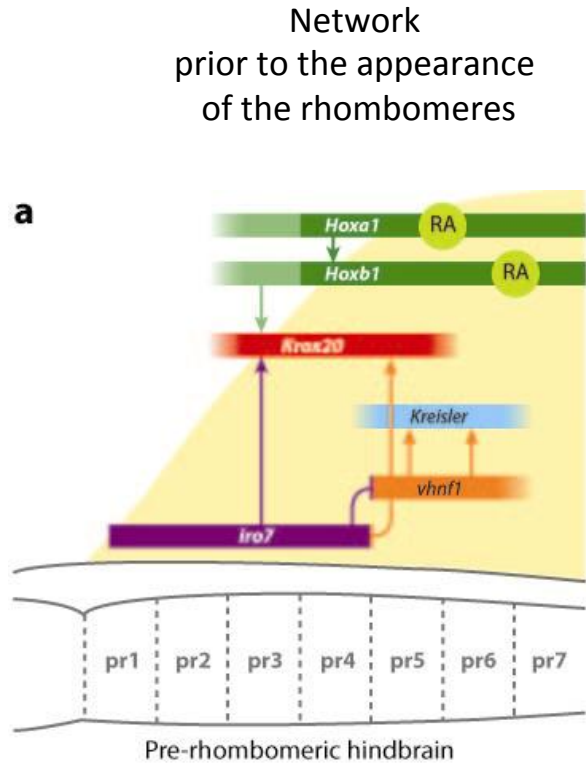


r1 is a hindbrain ground ("default") state

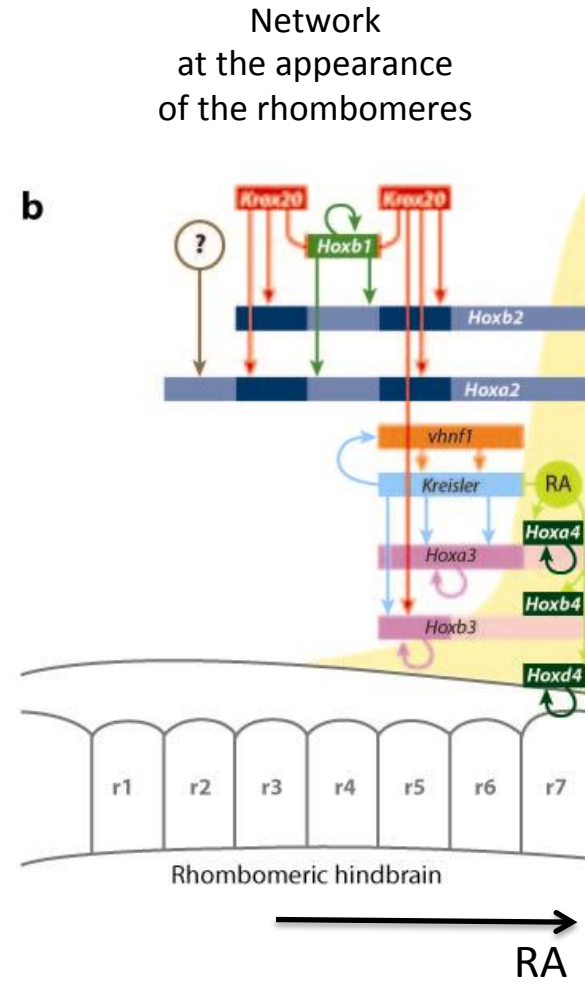


→ hindbrain neuron identity is homogenized in embryos lacking Pbx function

What control the patterned expression of Hox genes in vertebrates?

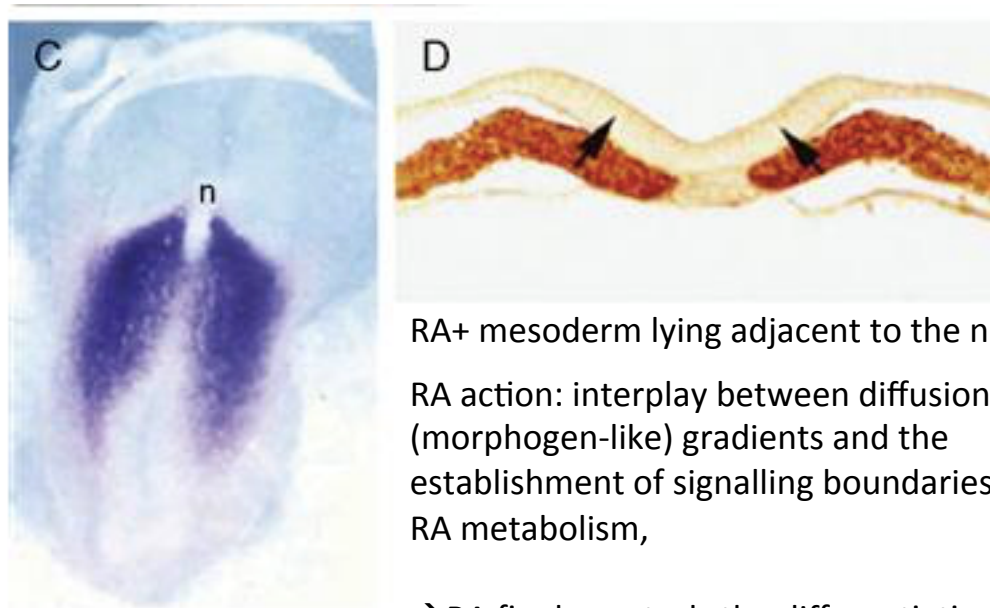


The yellow background represents the retinoic acid (RA) gradient



Gene patterning network of the vertebrate hindbrain

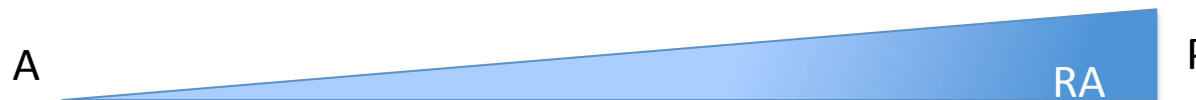
In normal embryos there is a gradient of RA expression (high levels in the posterior regions)



RA+ mesoderm lying adjacent to the neural tube

RA action: interplay between diffusion (morphogen-like) gradients and the establishment of signalling boundaries due to RA metabolism,

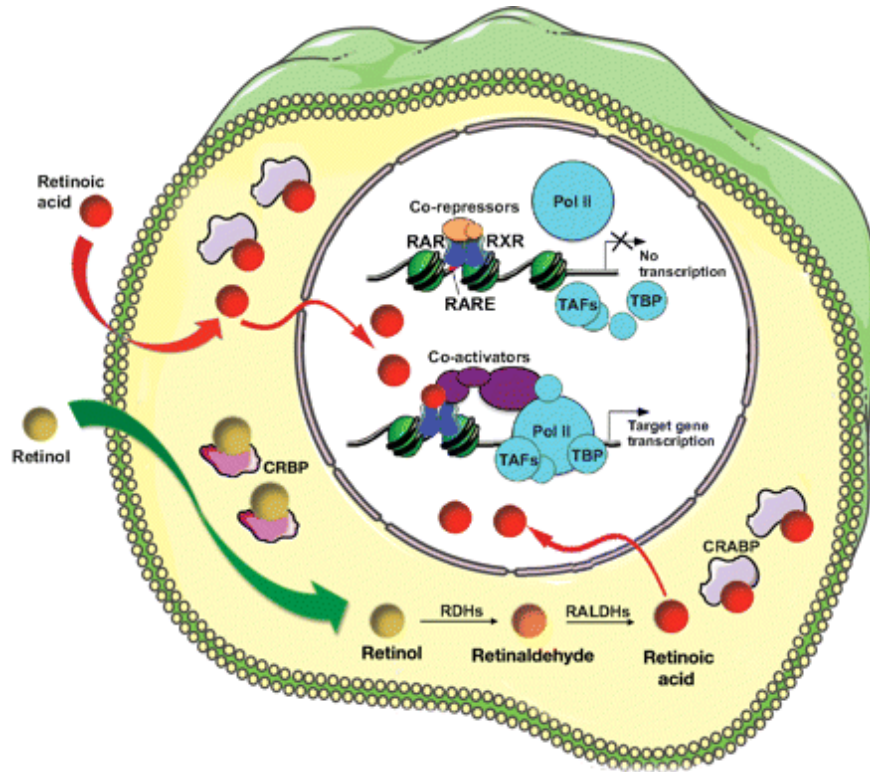
→RA finely controls the differentiation and patterning of various stem/progenitor cell populations



Hoxa1 – Hoxb1 promoters have RAREs (RA-response elements)

RA signalling pathway

RA is synthesized intracellularly from circulating retinol or diffuses from an adjacent cell (curved red arrow).

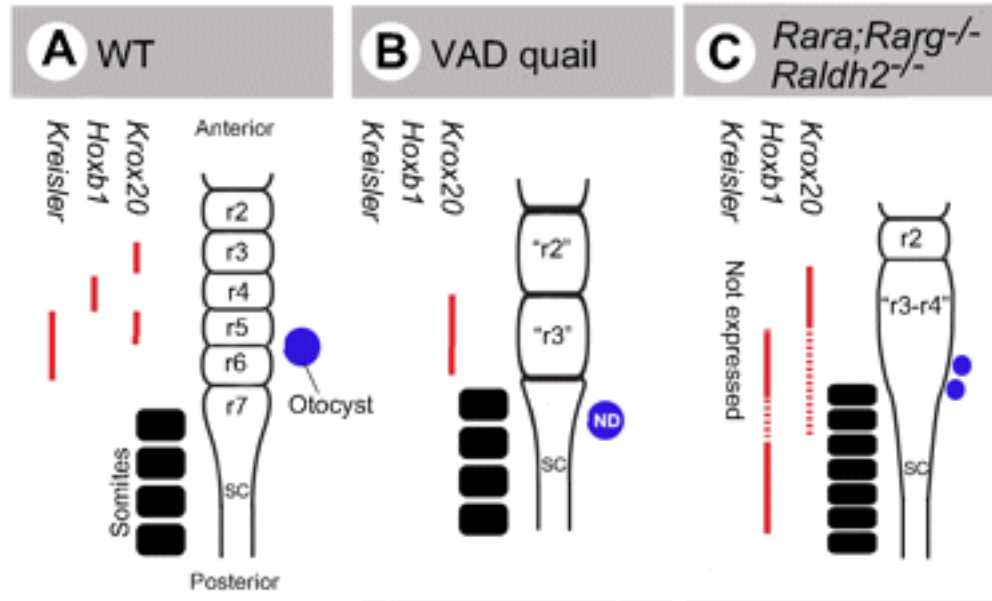


Rhinn et al., Development 2012

In absence of RA, dimers of RA receptors (RARs) and retinoid X receptors (RXRs), bind to RA-response elements (RAREs) in their target genes, interacting with protein complexes (co-repressors) that stabilise the chromatin nucleosomal structure and prevent access to the promoter.

Upon RA binding, a conformational change in the helicoidal structure of the RAR ligand-binding domain changes its protein-protein interaction properties, releasing the co-repressors and recruiting co-activator complexes that destabilise the nucleosomes and/or facilitate assembly of the transcription pre-initiation complex, which contains RNA polymerase II (Pol II), TATA-binding protein (TBP) and TBP-associated factors (TAFs).

Hindbrain abnormalities in animal models with altered RA signalling

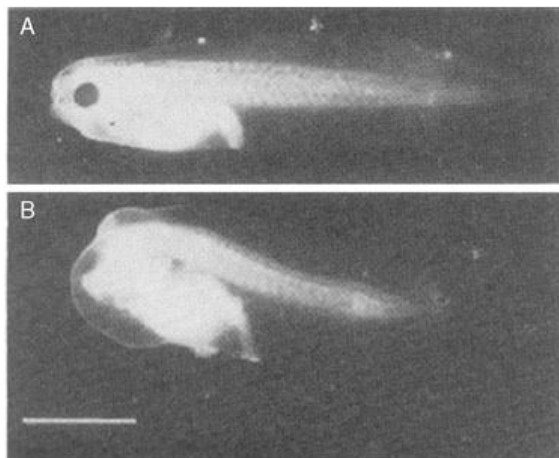


animal models **with endogenous deficiency in RA signalling:**

(B) vitamin A-deficient (VAD) **quail**;

(C) *Raldh2^{-/-}*, as well as *Rara^{-/-};Rarg^{-/-}* signalling mutant **mice** (lacking RAR α and RAR γ);

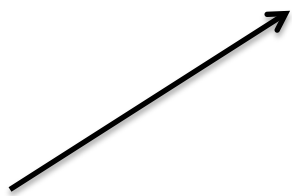
alteration in posterior brain development



← RA-treated Xenopus embryo

defects in anterior part of CNS

RA



INDUCTIVE SIGNALS

In vitro addition of RA to rapidly proliferating mouse ES cells, cultured in suspension as embryoid bodies, leads to the selective generation of neural progenitors with characteristics of radial glial cells found in the developing central nervous system



TERATOGENIC

Accutane (isotretinoin) a popular drug for severe acne introduced in the '80s

(Craniofacial and brain abnormalities)

In vertebrates Hox gene expression does not extend to the mesencephalon and prosencephalon...other homeobox containing gene families play a role in the development of these brain regions (e.g Dlx and Pax)

**Signaling molecules that patterns
the anterior-posterior axis in vertebrates**

→ early events in neural induction
influences the A-P axis

Regional specificity
of neural induction

Head-trunk-tail
organizer model

Transplantation of small regions of organizer tissues at different times during gastrulation results in induction of different parts of the neuraxis

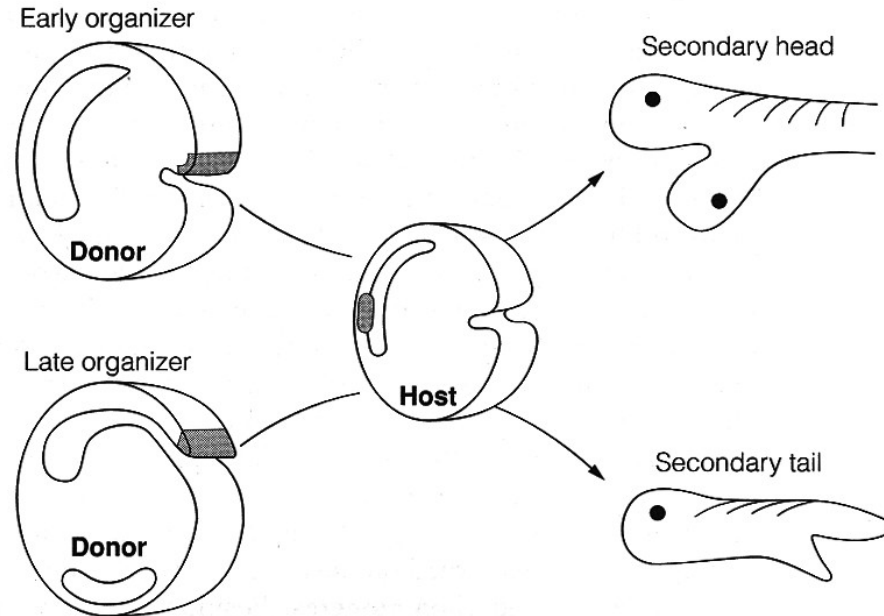


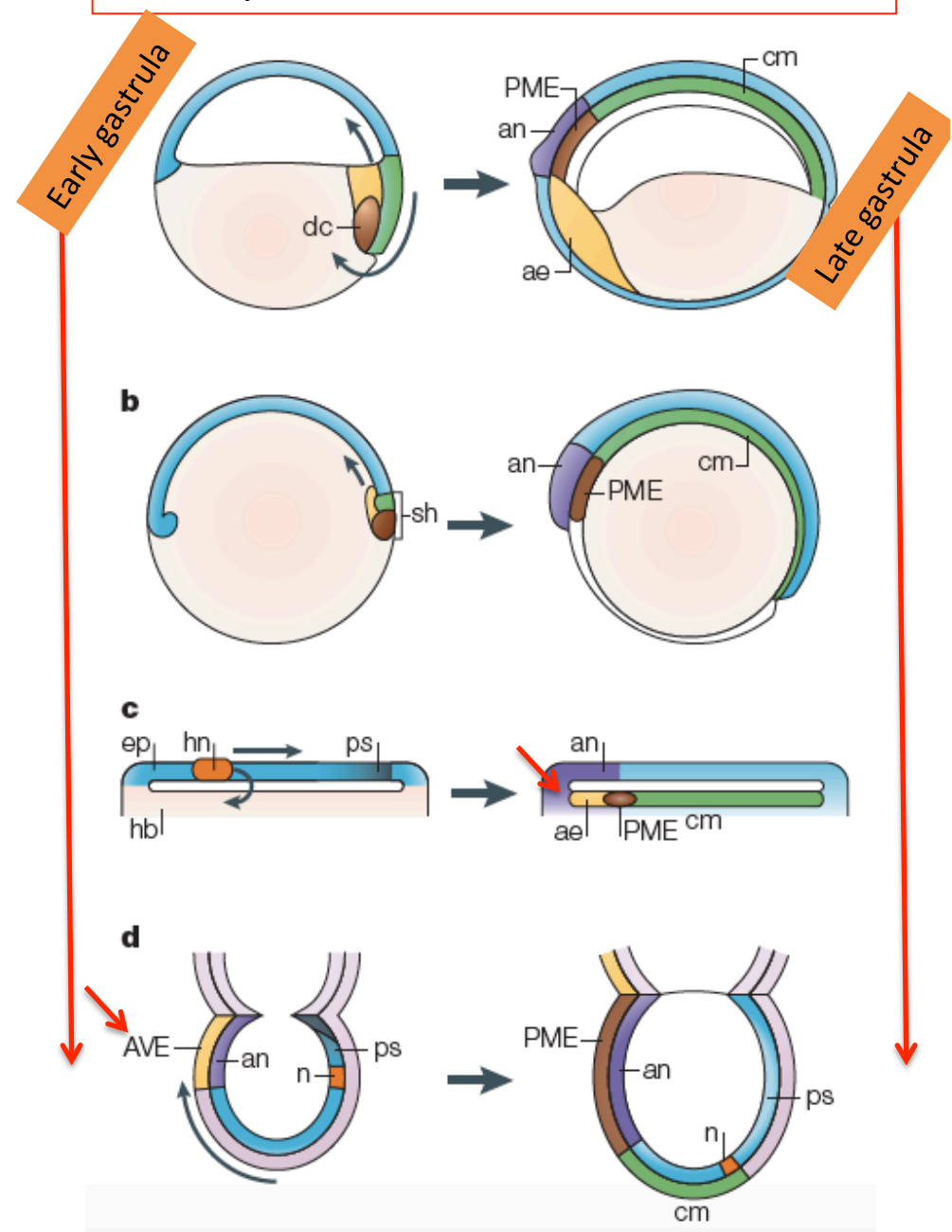
Fig. 2. Grafting experiments show the regional specificity of neural induction.

Early organizer= early gastrula lip=presumptive prechordal mesoderm PME --> **head organizer**

Late organizer=late gastrula lips=presumptive chordal mesoderm --> **tail organizer**

Figure 1 | **Comparative diagram of Spemann–Mangold organizer development in (a) *Xenopus laevis*, (b) zebrafish, (c) chick and (d) mouse gastrulae.** Left side, early gastrulae; right side, late gastrulae/early NEURULAE. Sagittal views are shown. The early gastrulae in a and b are shown with the ANIMAL POLE to the top, dorsal to the right. In all other panels, anterior points to the left, dorsal to the top. a | In *X. laevis*, the organizer is located in the upper dorsal blastopore lip. Its different cell populations are the leading edge cells, which give rise to ANTERIOR ENDODERM (ae; yellow). Prechordal mesendoderm (PME; brown) is derived from the deep cells (dc; brown) of the Spemann–Mangold organizer and underlies the anterior neural plate (an; purple) in the late gastrula. The last cells to involute are chordamesodermal cells (cm; green). b | In zebrafish, the organizer is located in the shield (sh), which contains the indicated cell populations. c | The chick embryo is a bilayered structure that is composed of the EPIBLAST (ep; blue) and the extraembryonic HYPOBLAST (hb; flesh coloured). At the onset of gastrulation, a full-length PRIMITIVE STREAK (ps) with Hensen’s node (hn; the chick organizer; orange) at its tip has formed. Both contain precursors of PME and chordamesoderm. During gastrulation, cells ingress through the node, form the PME and chordamesoderm and displace the hypoblast anteriorly. d | In the mouse, the equivalent of the Spemann–Mangold organizer is located in the primitive streak and Hensen’s node. A supporting signalling centre resides in the anterior visceral endoderm (AVE; yellow), which juxtaposes the prospective anterior neural plate. The primitive streak with the node (n; the mouse organizer; orange) forms at the posterior end of the embryo. Similar to the chick, both streak and node contain precursors of PME and chordamesoderm. The streak elongates during gastrulation while cells emigrate through the node and form the axial mesendoderm that displaces the AVE. At the end of gastrulation, the PME underlies the anterior neural plate and is followed posteriorly by chordamesoderm. Modified with permission from REF. 20 © (2001) Elsevier Science Ltd.

The organizer is not a homogenous tissue but a dynamic structure...



Nieuwkoop model of neural patterning

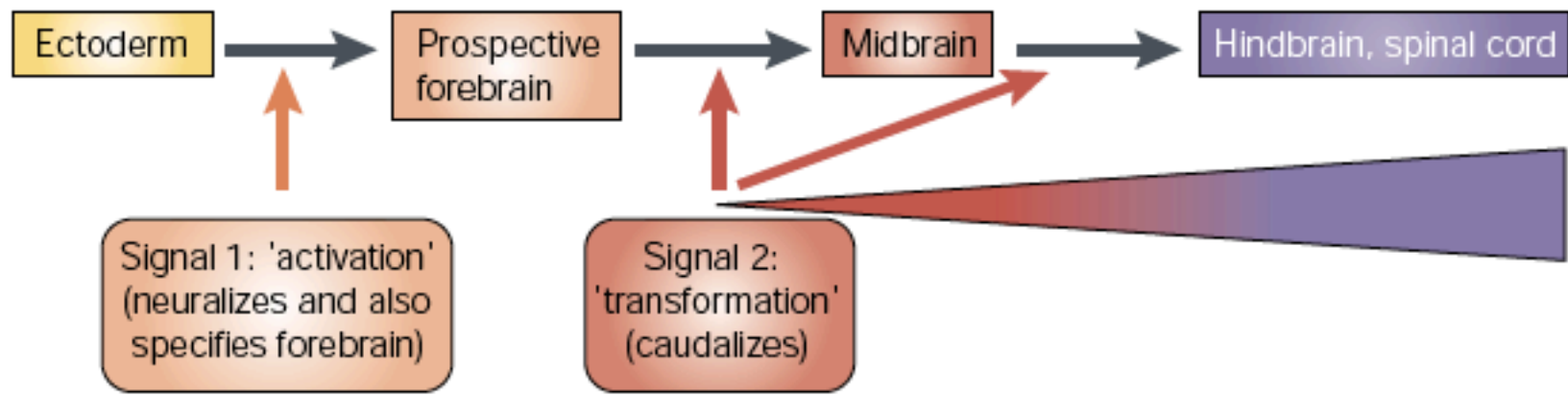
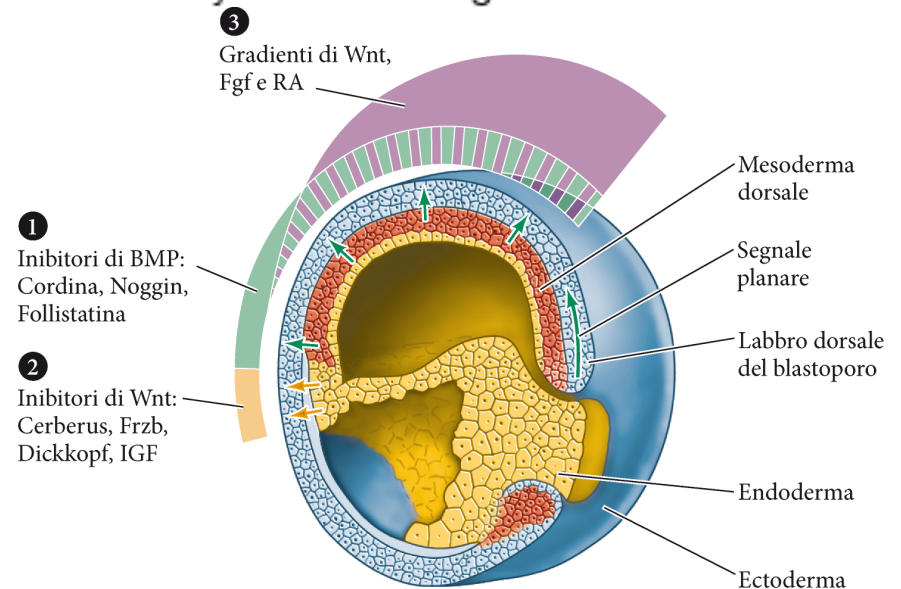


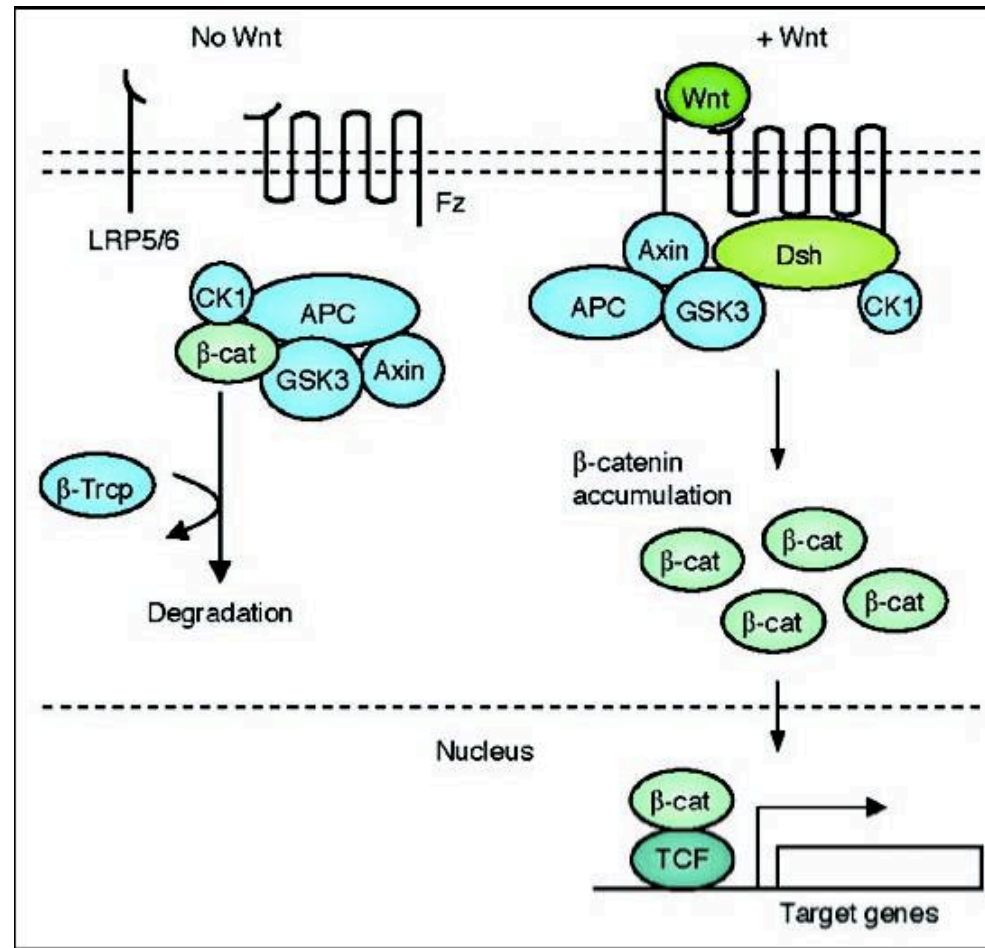
Figure 3 | **Nieuwkoop's activation-transformation model**⁴⁸. Induction of the nervous system is proposed to occur in two steps: initial induction of forebrain, and subsequent caudalization of part of it. According to this model, the organizer emits both the activating and the transforming signals. The gradient represents either increasing strength of the transforming signal, or increasing time of exposure to this signal, which results in portions of the nervous system becoming progressively more caudal.

Activation: **Noggin, Chordin, Follistatin**

Transformation: **RA, Wnt, FGF**



Wnt signaling pathway



Komiya et al., 2008 Organogenesis

One key level of regulation of Wnt signaling occurs in the extra-cellular milieu with the presence of secreted Wnt antagonists

Cerberus; Dickkopf; FrzB

Co-inhibition of Wnt and BMP signals lead to induction of anterior neural structures

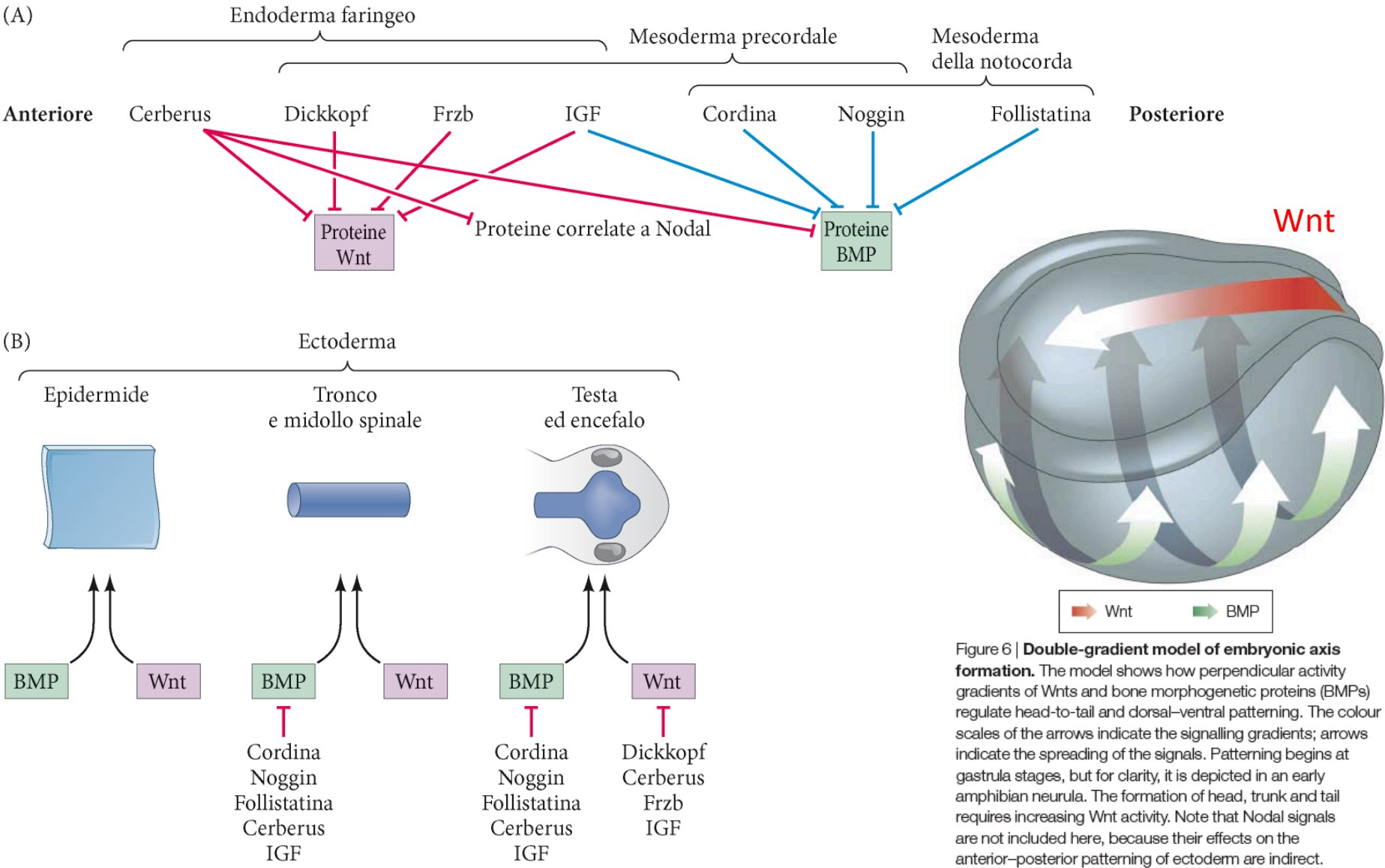
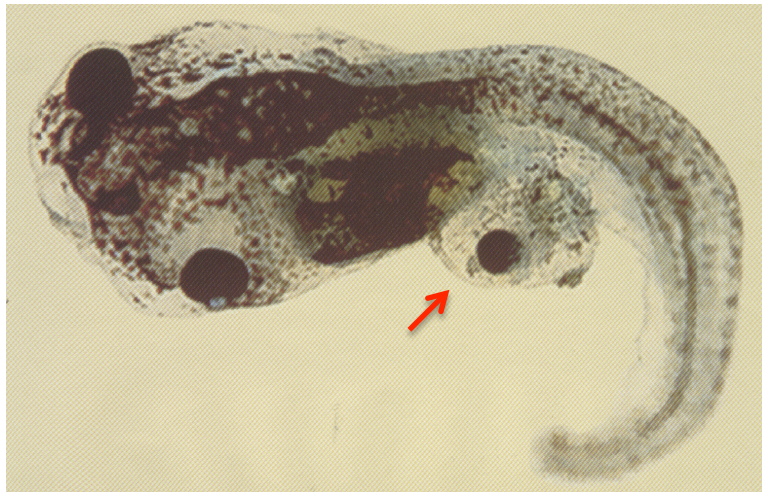


Figure 6 | **Double-gradient model of embryonic axis formation.** The model shows how perpendicular activity gradients of Wnts and bone morphogenetic proteins (BMPs) regulate head-to-tail and dorsal-ventral patterning. The colour scales of the arrows indicate the signalling gradients; arrows indicate the spreading of the signals. Patterning begins at gastrula stages, but for clarity, it is depicted in an early amphibian neurula. The formation of head, trunk and tail requires increasing Wnt activity. Note that Nodal signals are not included here, because their effects on the anterior-posterior patterning of ectoderm are indirect. Modified with permission from REE. 78 © (2001) The Company of Biologists Ltd.

Cerberus

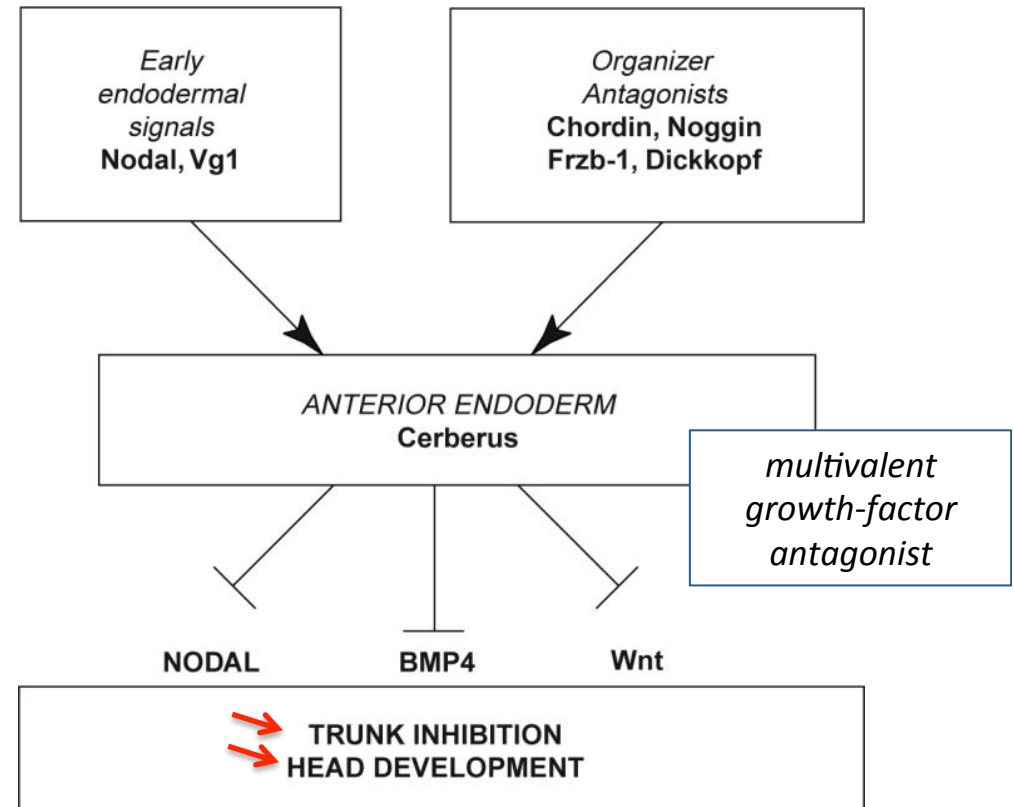
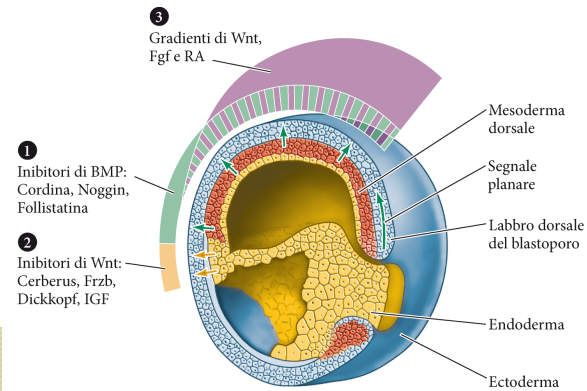
Expressed in:

- anterior primitive endoderm
- pre-chordal mesoderm



Microinjection of cerberus mRNA into *Xenopus* embryos induces ectopic heads in absence of trunk structures

In order for the head territory to form, the signals involved in trunk development must be inhibited in rostral regions



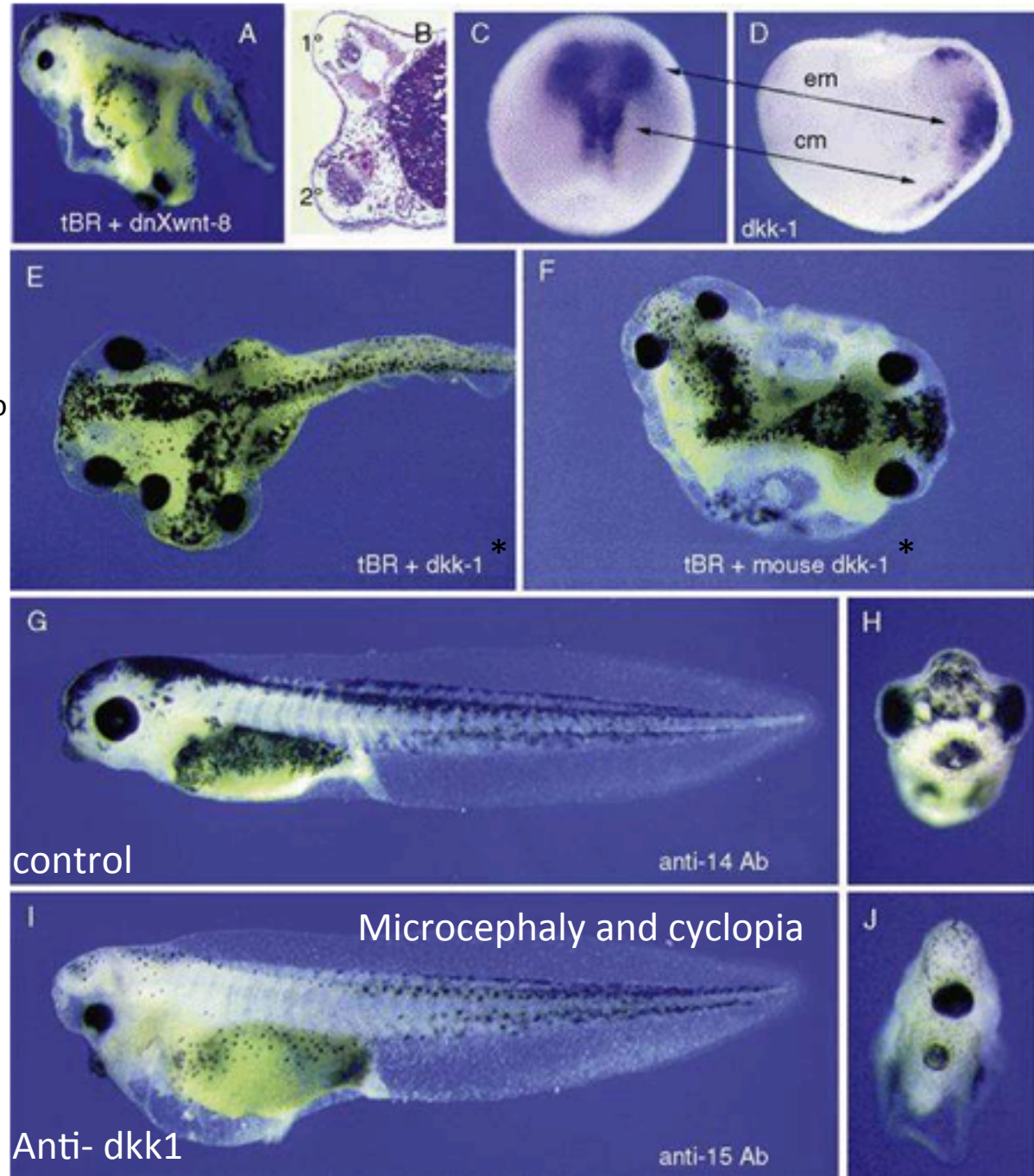
Dickkopf

Antagonism of Wnt and BMP signalling leads to induction of anterior neural structures

A,E,F Injection of a four cell Xenopus embryo

*high conservations among evolution for these proteins

tBR=truncated BMP receptor
 dnXwnt-8=dominant negative form of Wnt8
 Em=endomesoderm
 Cm= chordamesoderm



control

anti-14 Ab

Microcephaly and cyclopia

Anti- dkk1

anti-15 Ab

Dickkopf

Dkk KOs lack head and brain structures anterior to hindbrain

similar phenotype in Dkk+/-noggin+/- mutants

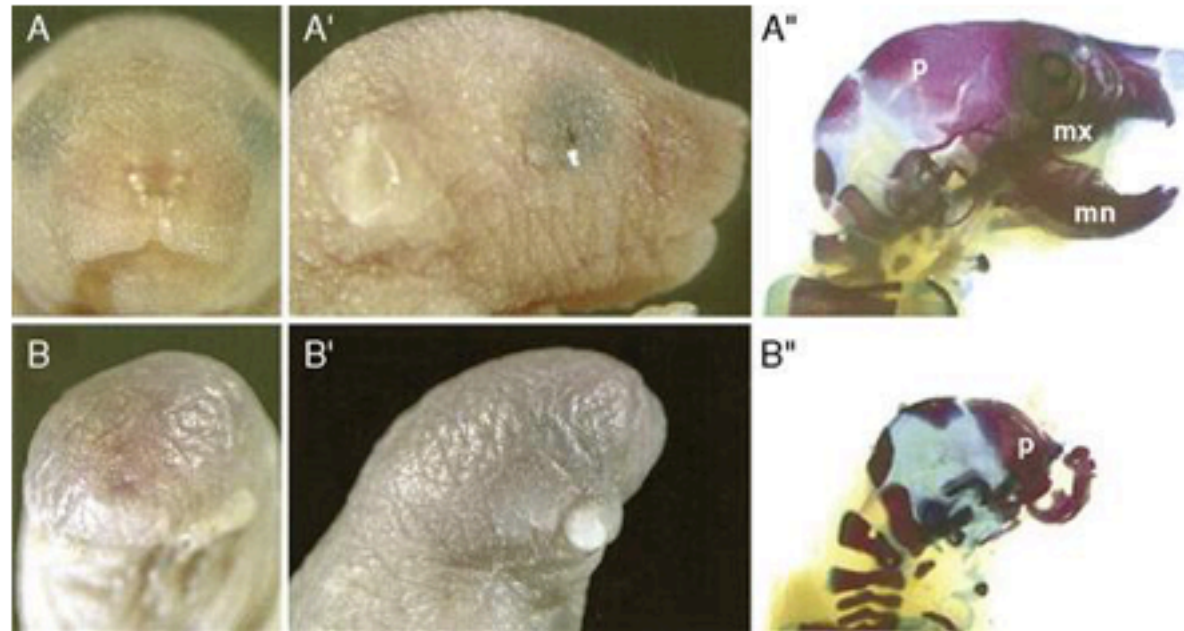


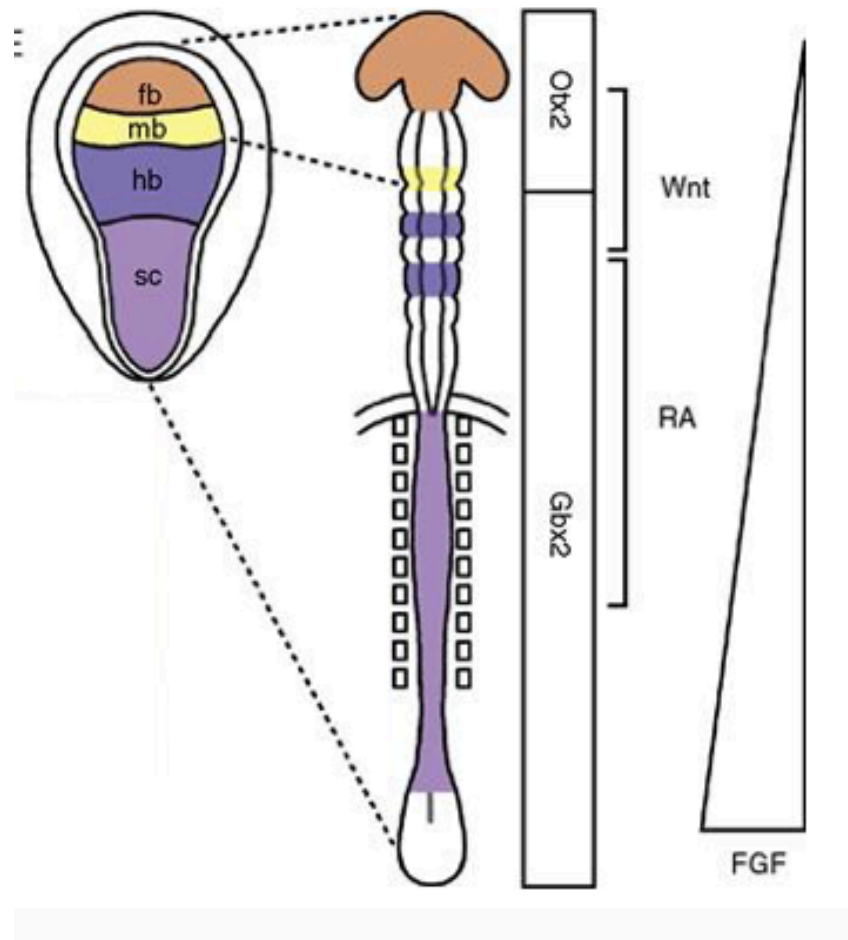
Fig. 2.10 *Dkk1* and *noggin* cooperate in head induction. Mice in which one allele for the genes for both *dkk1* and *nog* have been deleted have severe head defects. Frontal (*A,B*) and lateral (*A',B'*) views of wild-type (*A,A'*) and mutant (*B,B'*), newborn animals. Lateral view of skeletal preparations from wild-type (*A''*) and severe mutant (*B''*) newborn heads reveal loss of maxillar (*mx*), mandibular (*mn*), and other bones anterior to the parietal bone (*p*). Modified from [del Barco Barrantes et al., 2003](#)

FGF

-neural induction

-activates posterior genes in the neural tube

-acts in a concentration dependent manner



Integration of 3 transformer signals (RA,Wnt,FGF)