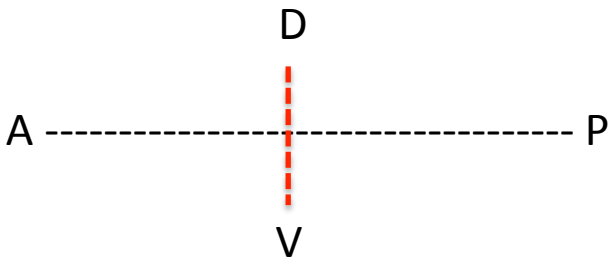
Patterning of the neural tube



- Within the neural tissue, the wide array of neurons and glial cells must be produced in a highly organized **temporal** and **spatial** order to form a functional nervous system

→ early events in neural induction influences the A-P axis: the early neural plate is already specified to form different parts of the nervous system as it arises following neural induction.

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

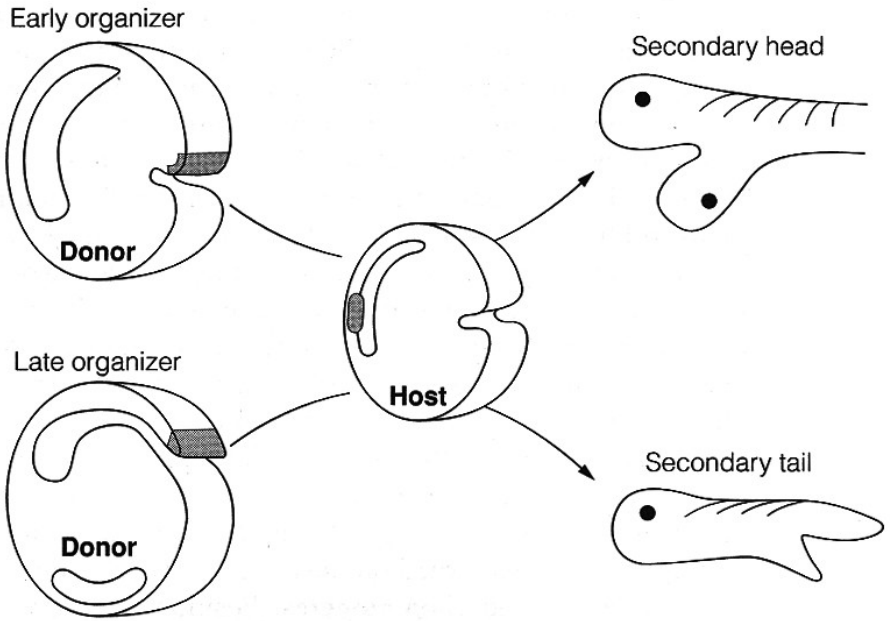


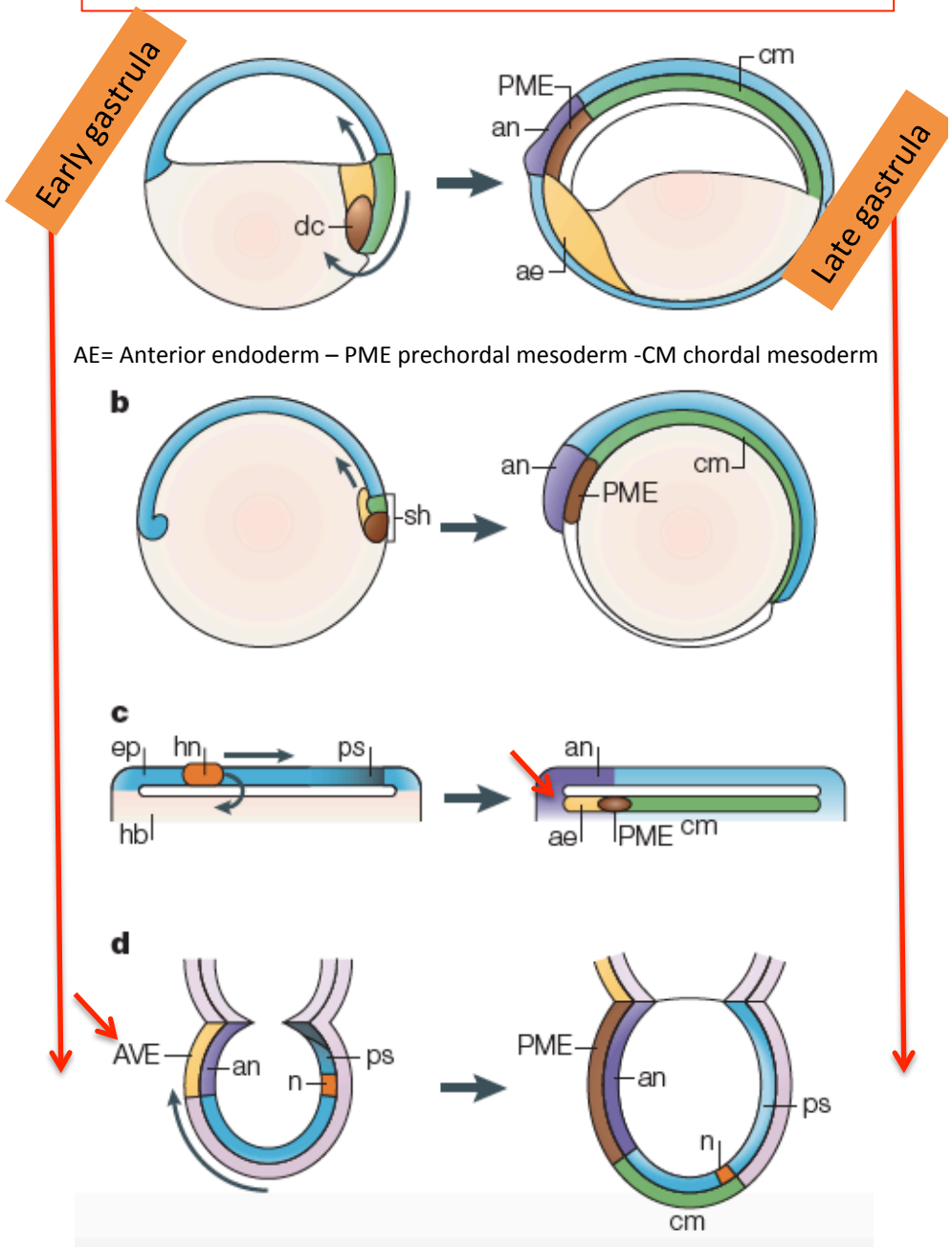
Fig. 2. Grafting experiments show the regional specificity of neural induction.

*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

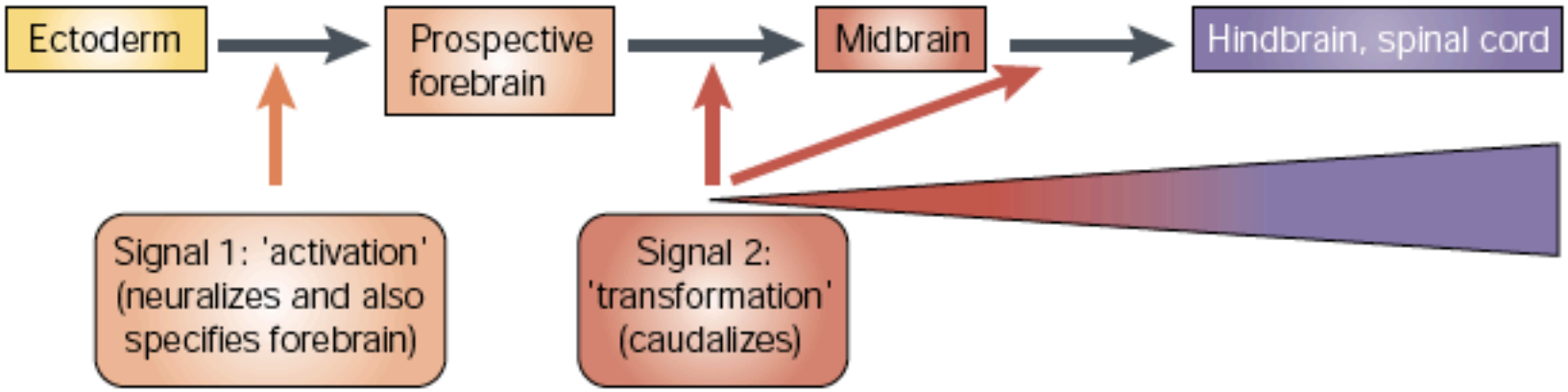
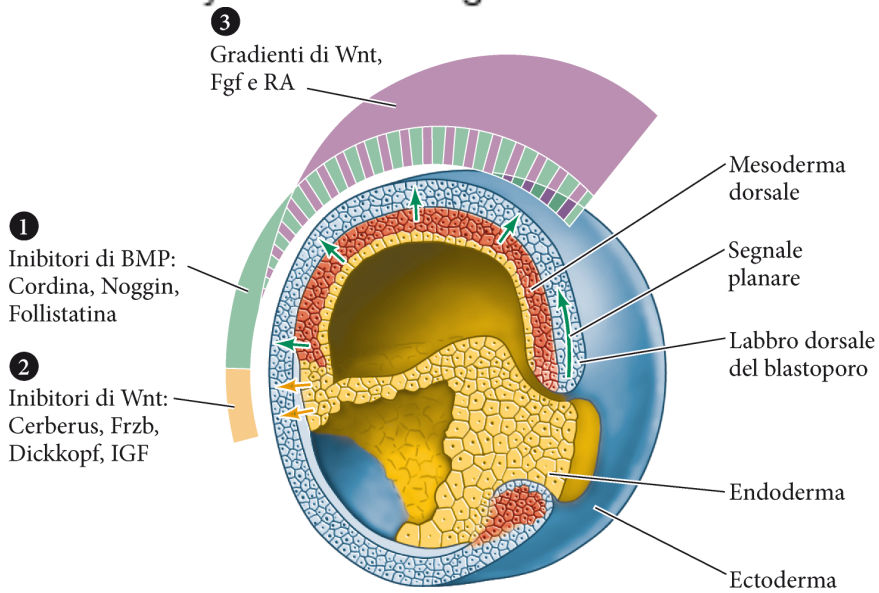


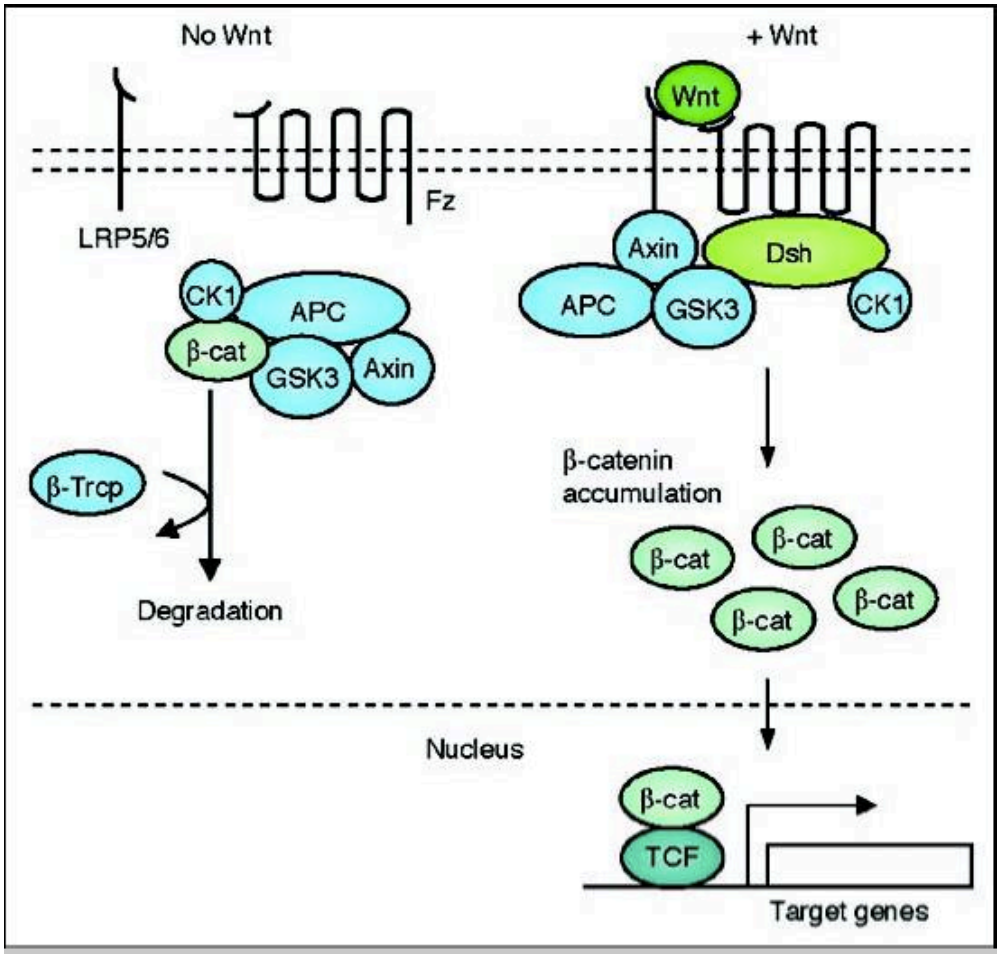
Figure 3 | **Nieuwkoop's activation–transformation model**[48]. Induction of the nervous system is proposed to occur in two steps: initial induction of forebrain, and subsequent caudalization of part of it. According to this model, the organizer emits both the activating and the transforming signals. 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

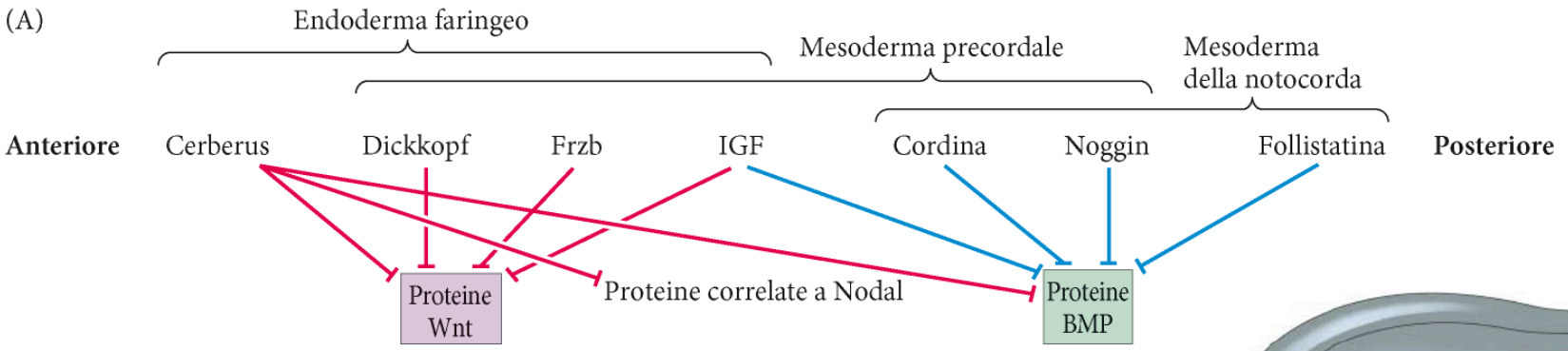


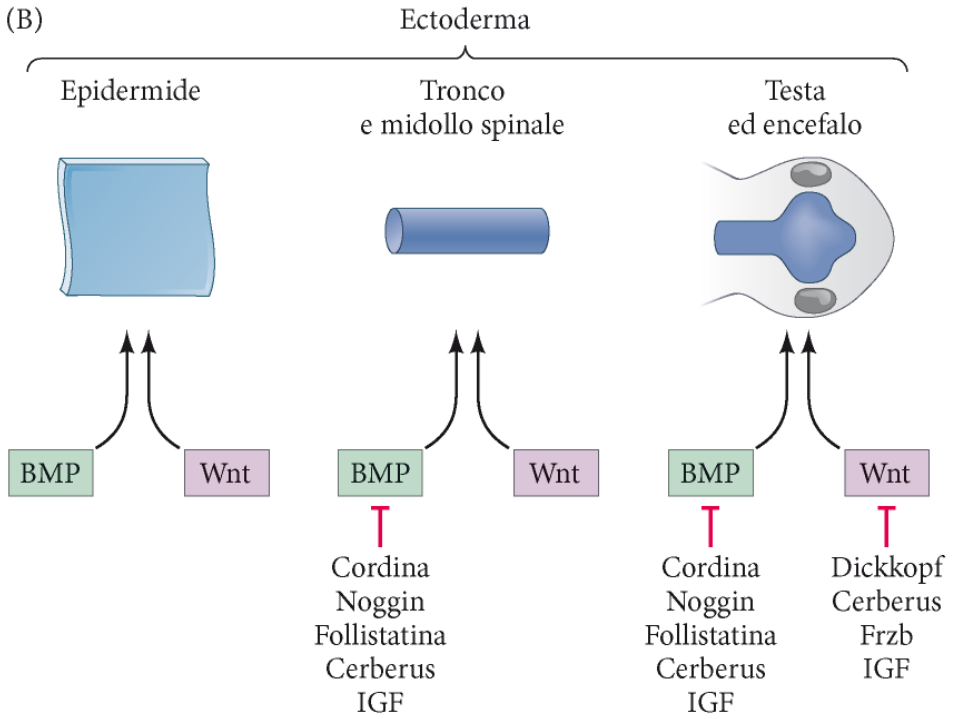
Komiya et al., 2008 Organogenesis

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





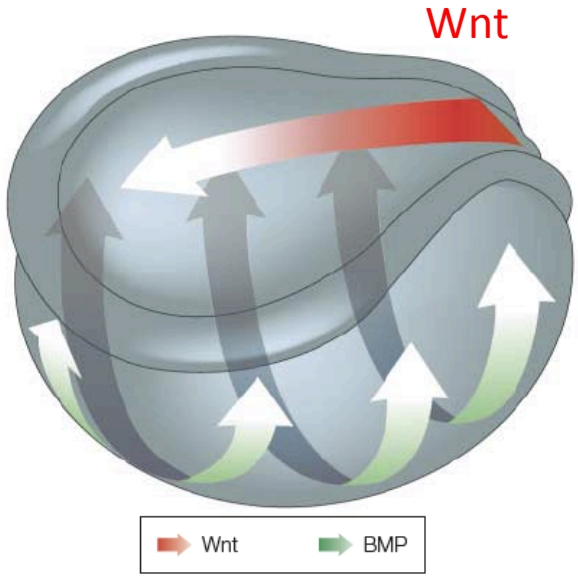
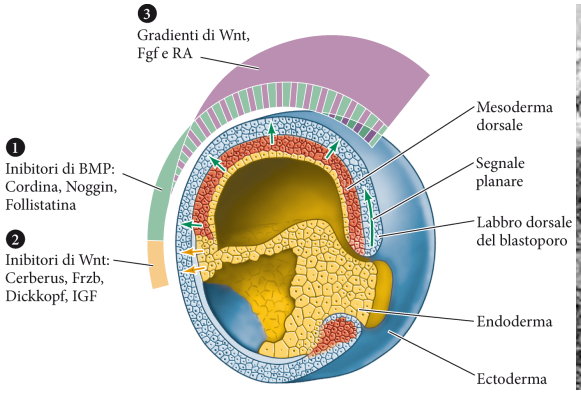


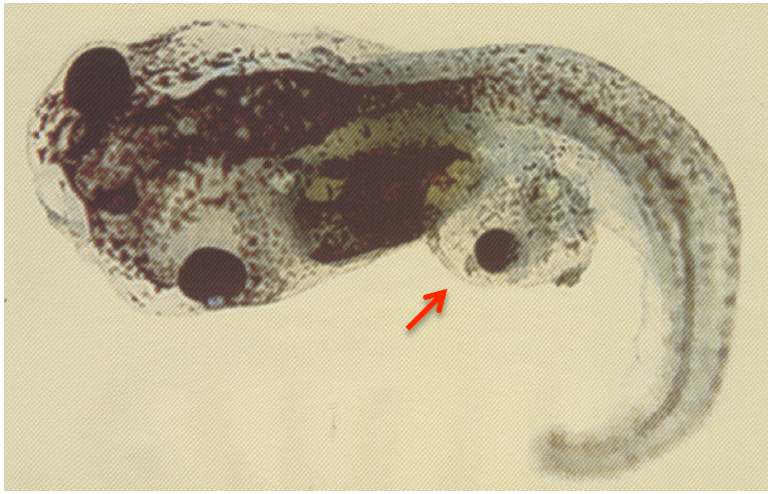
Figure 6 | **Double-gradient model of embryonic axis formation.** The model shows how perpendicular activity gradients of Wnts and bone morphogenetic proteins (BMPs) regulate head-to-tail and dorsal–ventral patterning. The colour scales of the arrows indicate the signalling gradients; arrows indicate 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 REF. 78 © (2001) The Company of Biologists Ltd.

Cerberus

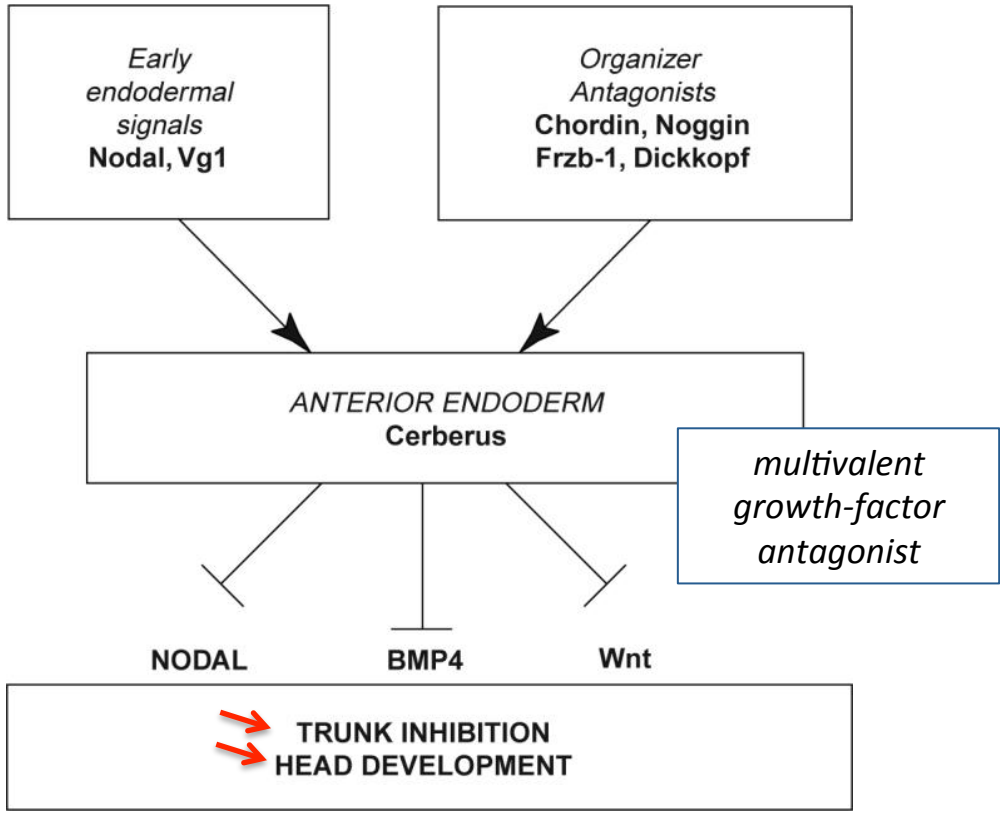
Expressed in:

- anterior primitive endoderm
- pre-chordal mesoderm





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

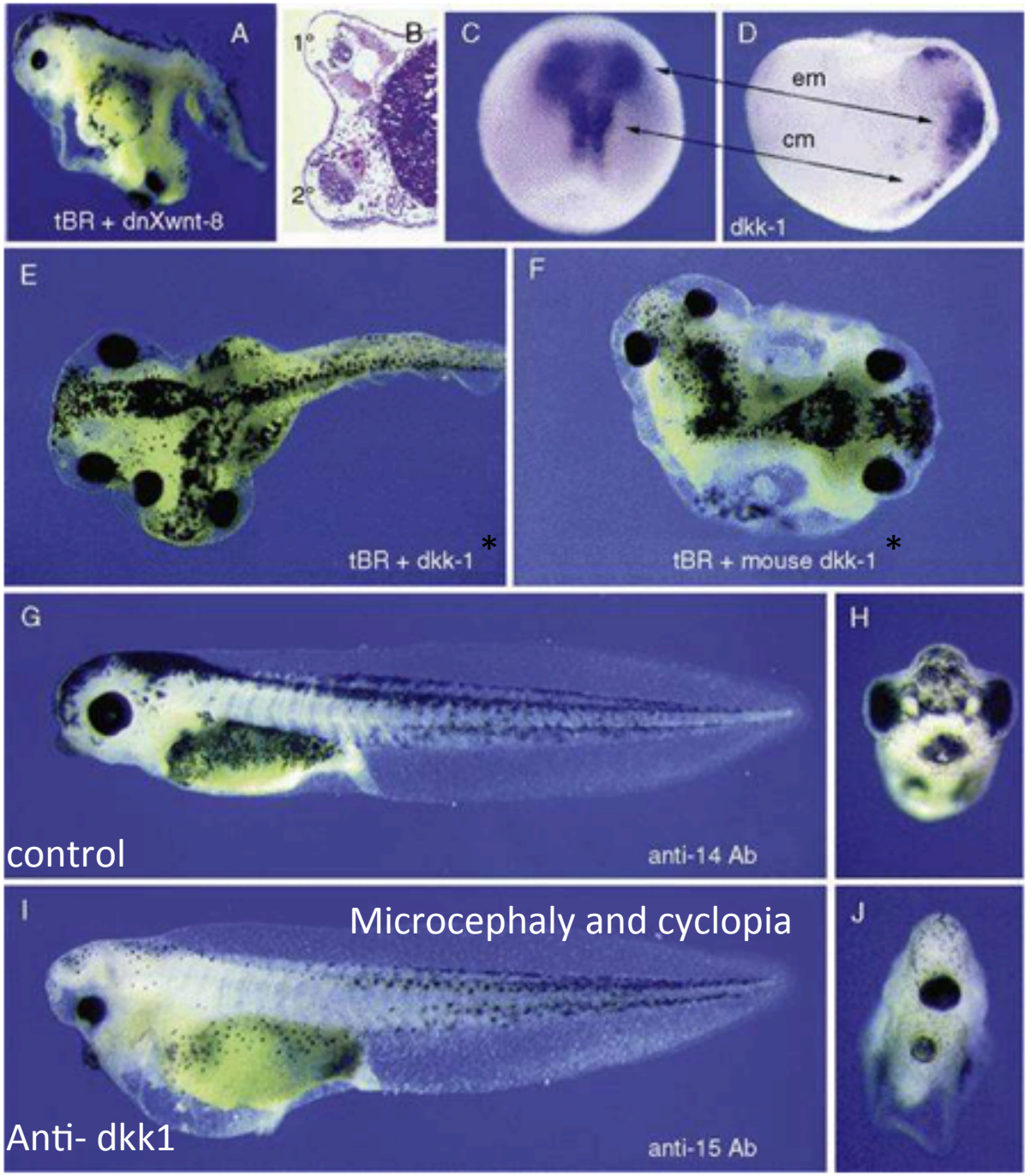


In order for the head territory to form, the signals involved in trunk development must be inhibited in rostral regions

Dickkopf

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

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



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

Dickkopf

Dkk KOs lack head and brain structures anterior to hindbrain

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

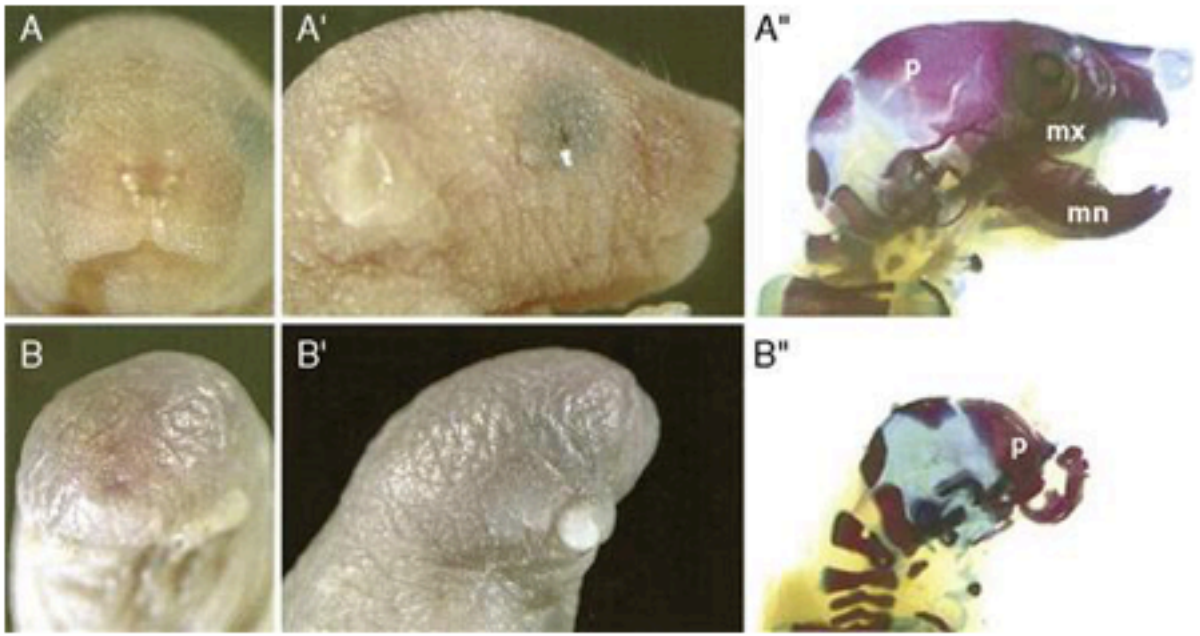
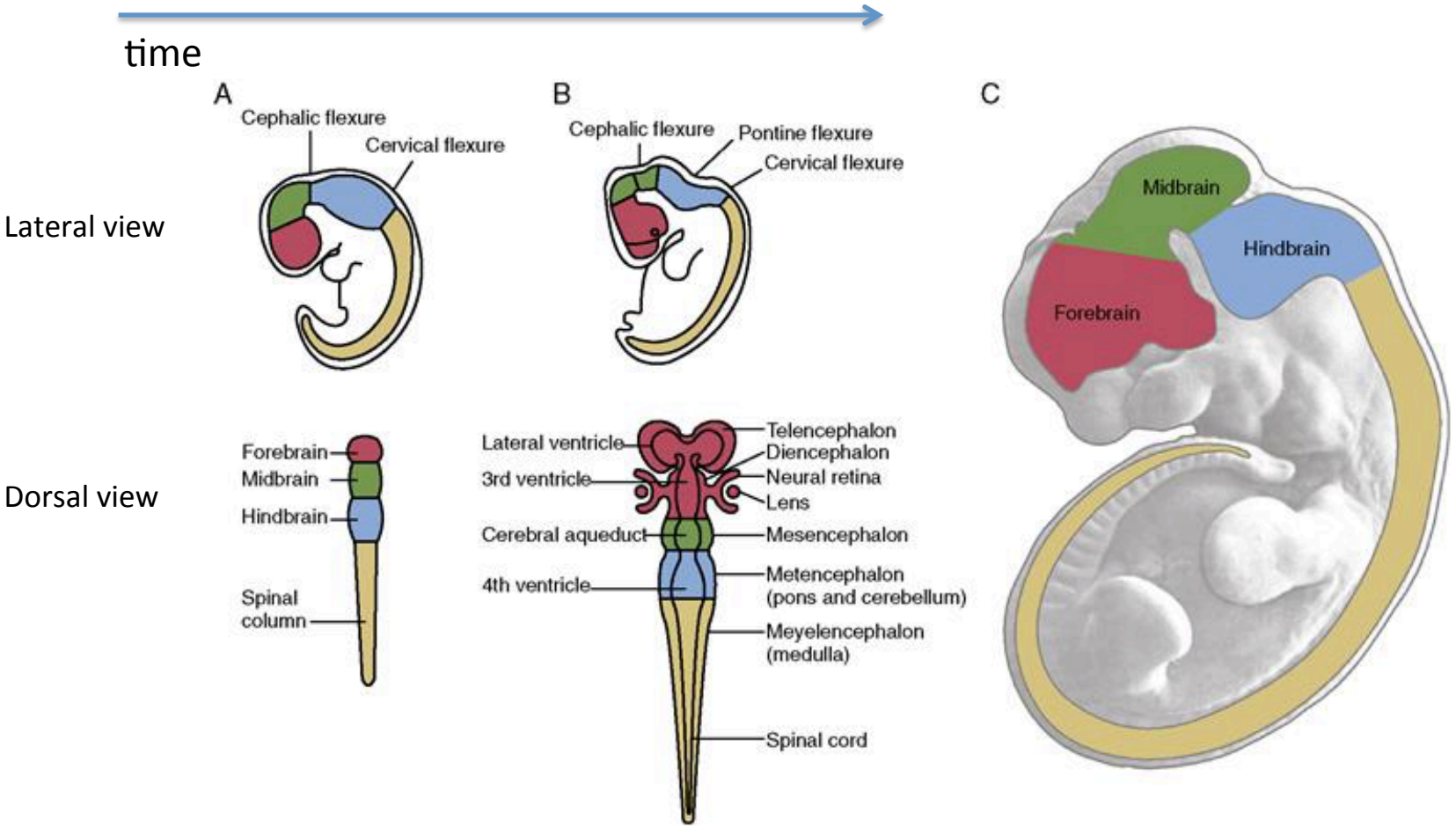
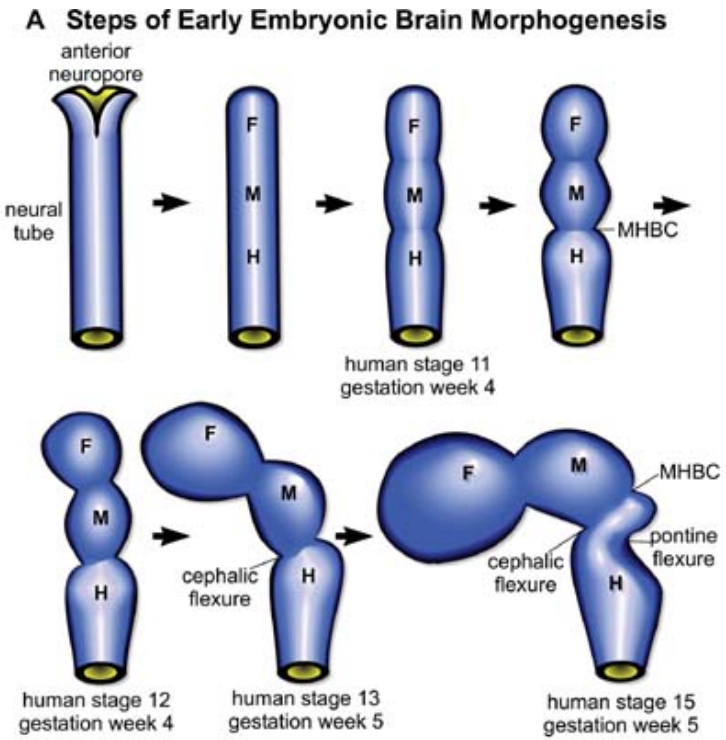


Fig. 2.10 *Dkk1* and *noggin* cooperate in head induction. Mice in which one allele for the genes for both *dkk1* and *nog* have been deleted have severe head defects. Frontal (*A*,*B*) and lateral (*A′*,*B′*) views of wild-type (*A*,*A′*) and mutant (*B*,*B′*), newborn animals. Lateral view of skeletal 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

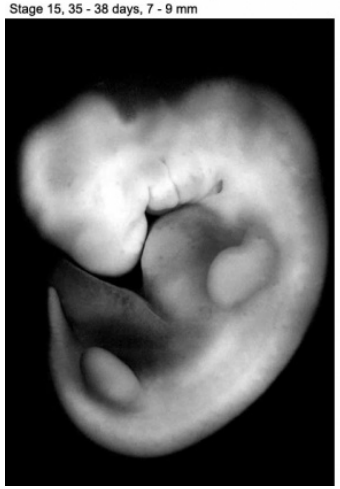
The nervous system is regionally specified during development



Human embryos (Sanes)

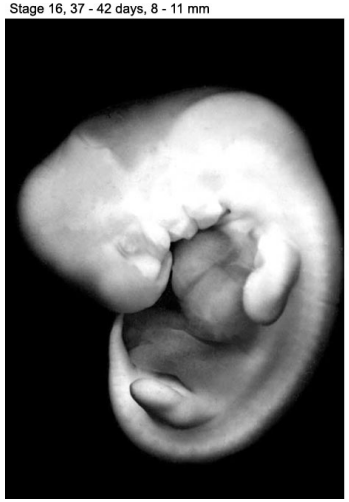


Embryo
Crown Rump Length (CRL) 9 mm
Stage 15, 35 - 38 days, 7 - 9 mm



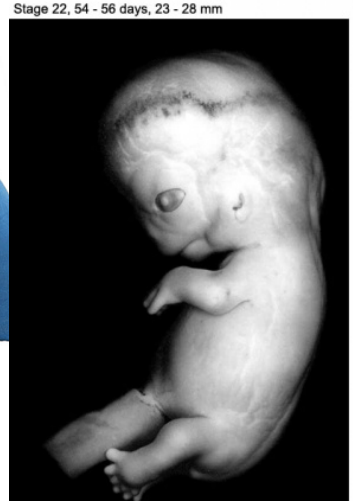
Neural Tube
Week 5

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

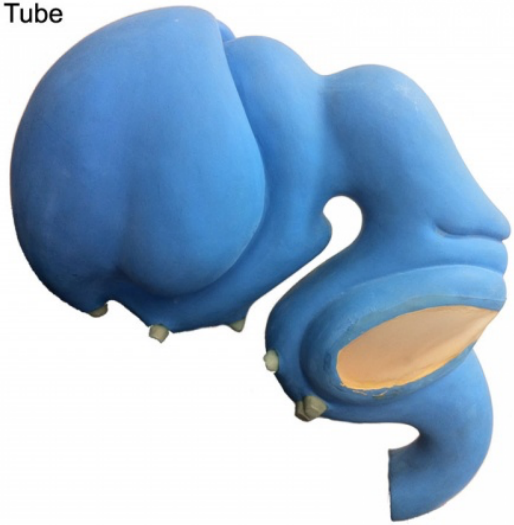


Neural Tube
Week 6

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

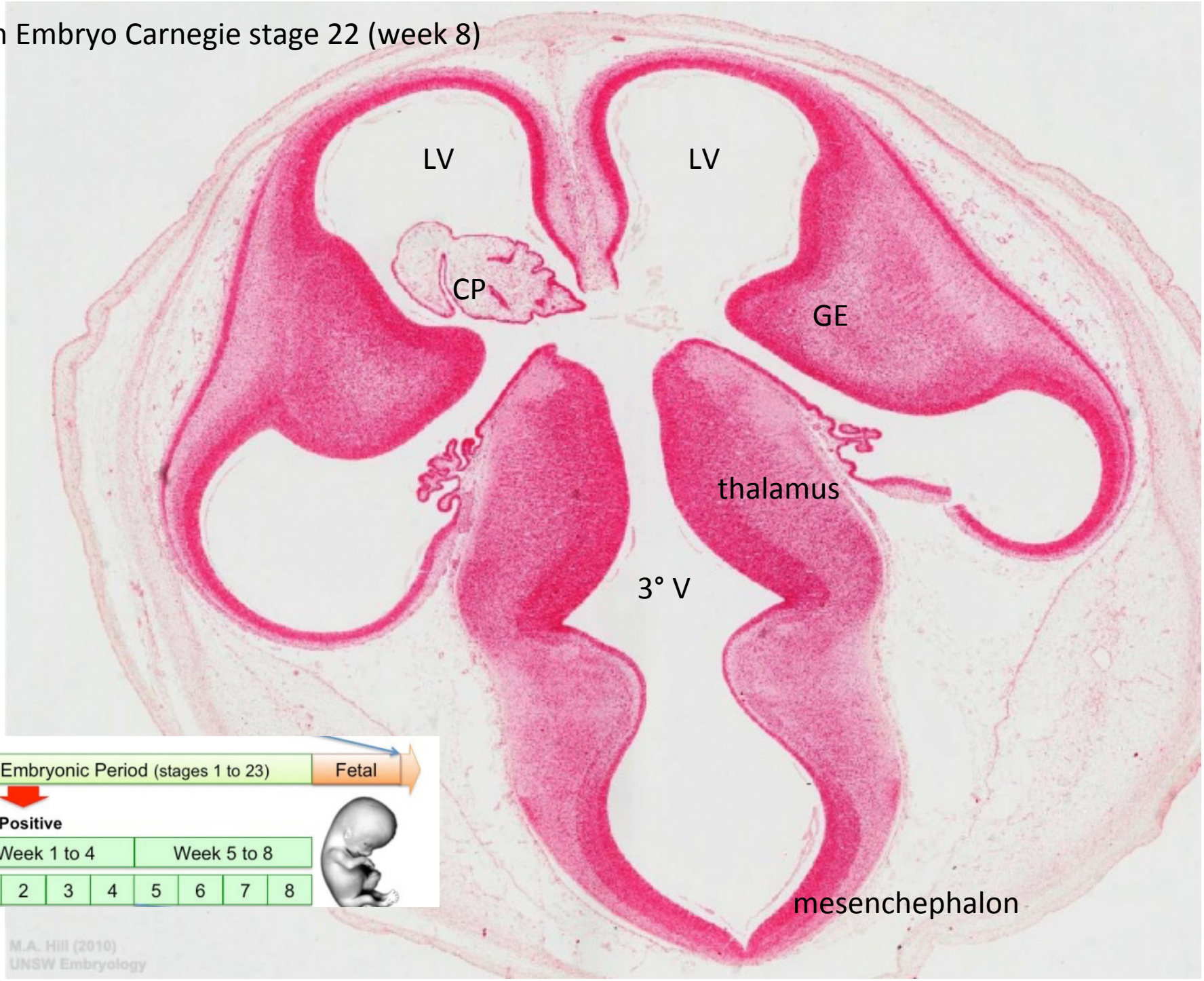


Neural Tube
Week 8

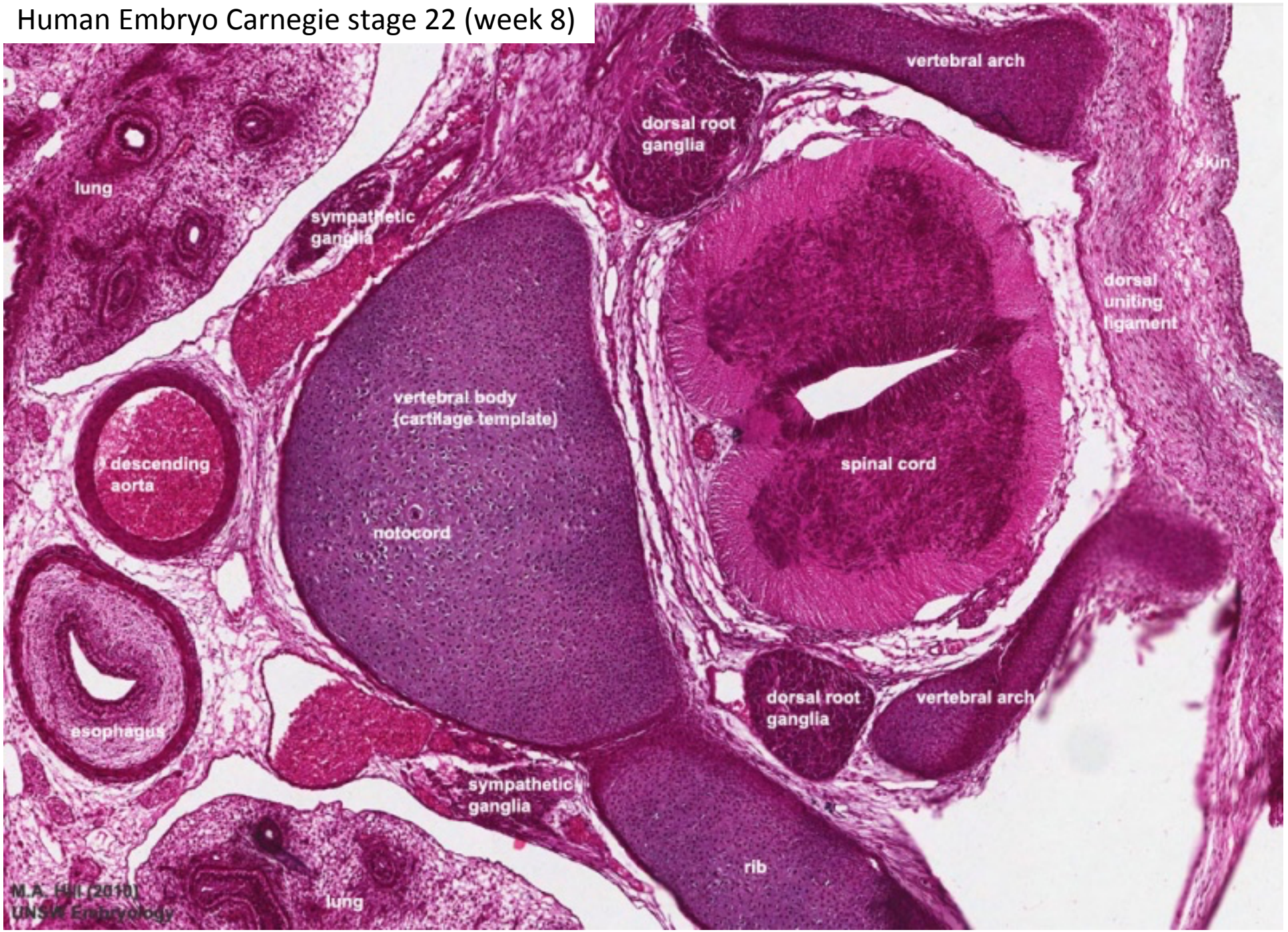


MHBC= Midbrain hindbrain boundary constriction

Human Embryo Carnegie stage 22 (week 8)

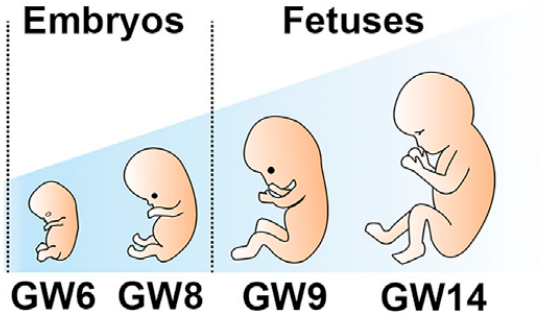


Human Embryo Carnegie stage 22 (week 8)



Tridimensional Visualization and Analysis of Early Human Development

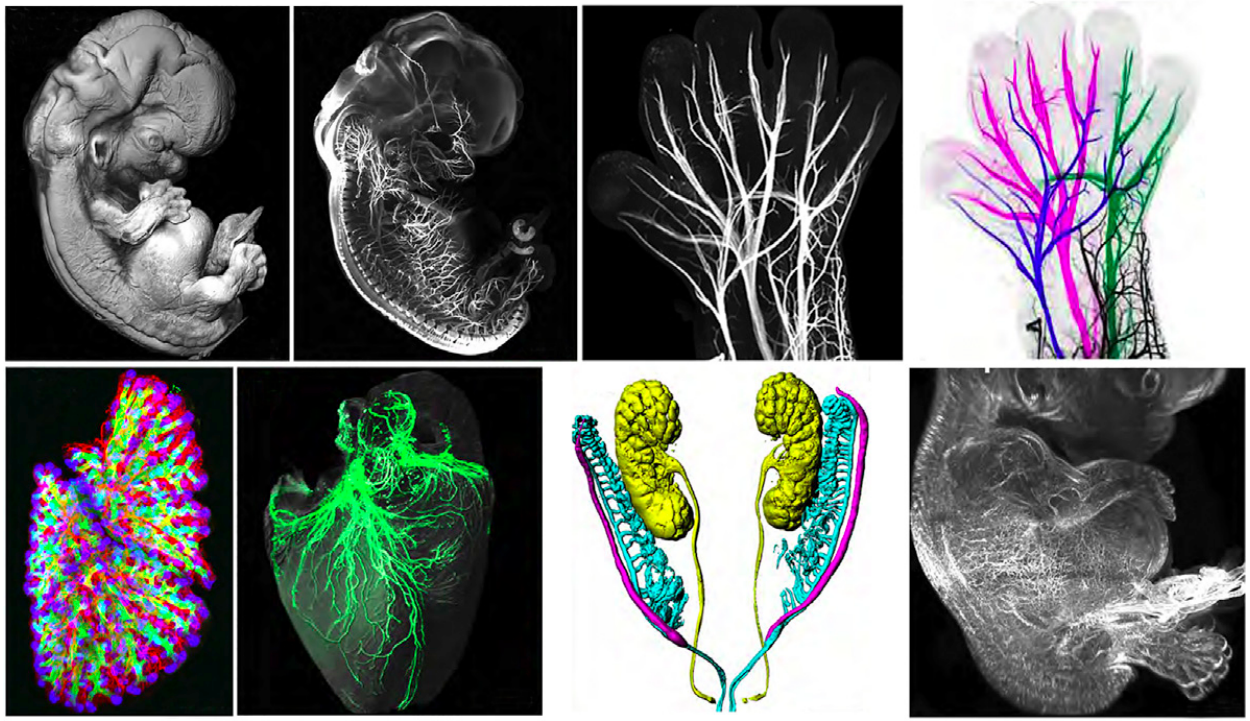
Collection of human embryonic and fetal specimens



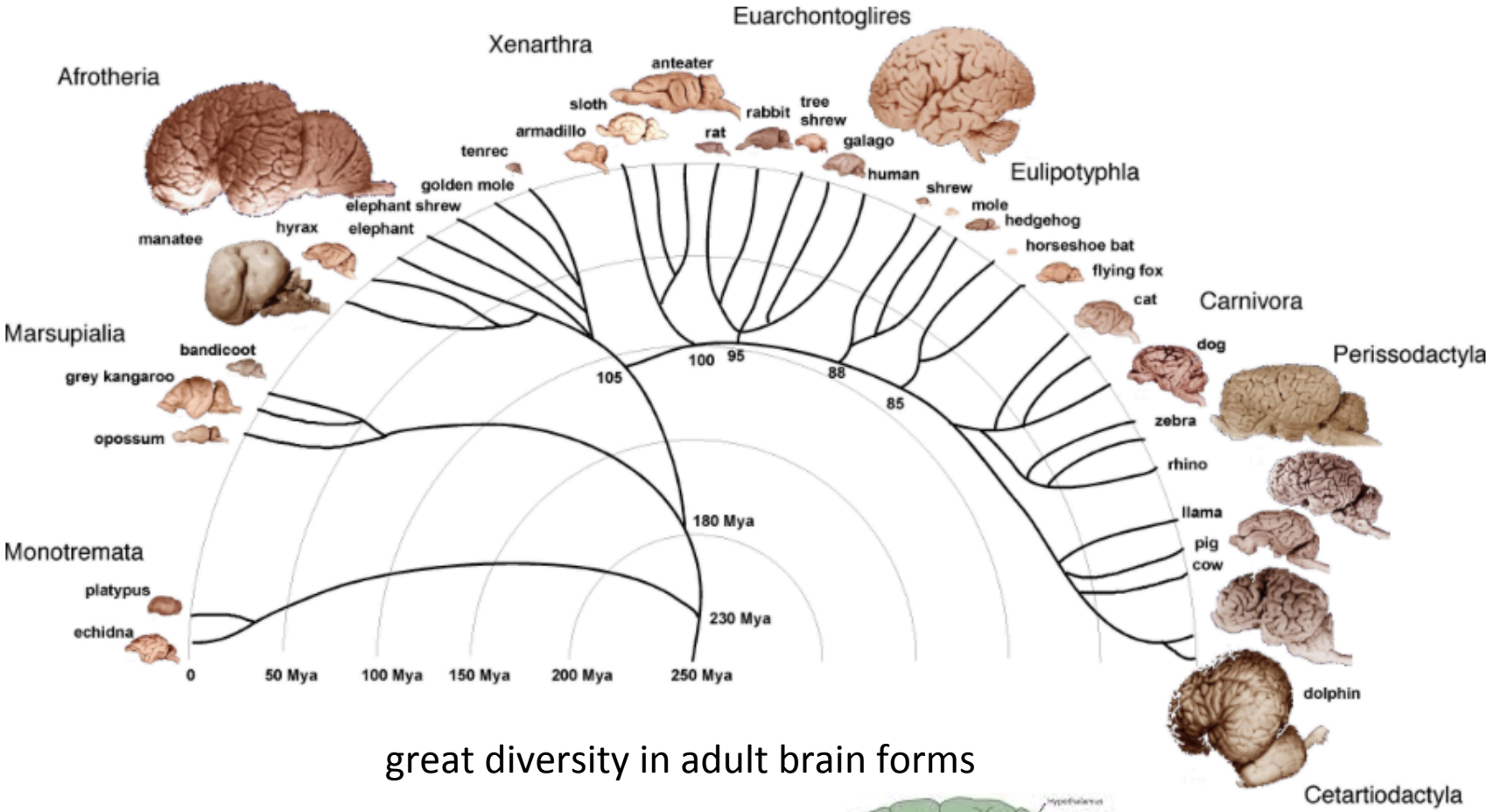
Whole-mount immunostaining 3DISCO clearing

Light-sheet microscopy

3D-Atlas of immunolabelled transparent human embryos/fetuses

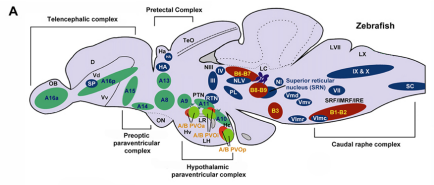


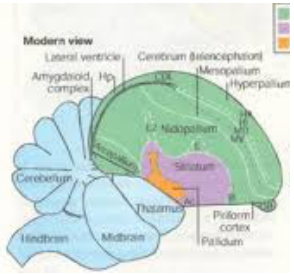
https://transparent-human-embryo.com

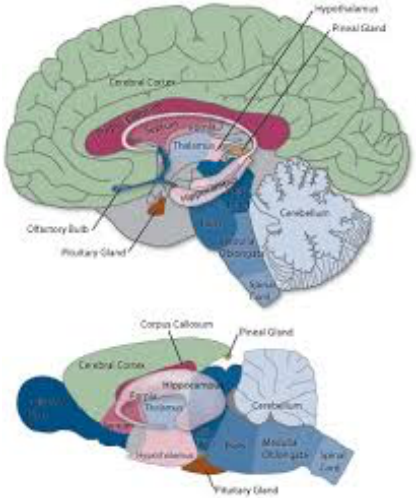


great diversity in adult brain forms

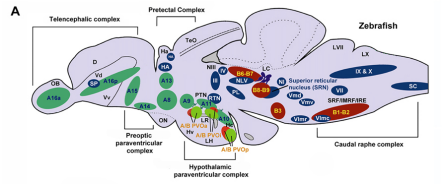
From Herculano-Houzel (2012) PNAS

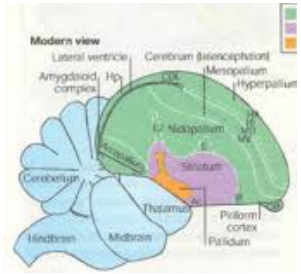


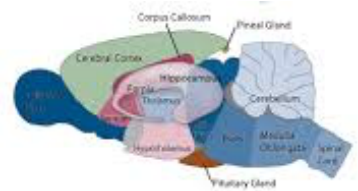


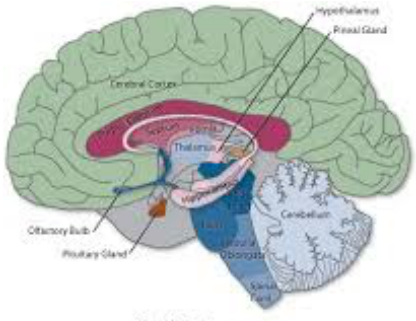


….early development of most vertebrate brains is similar









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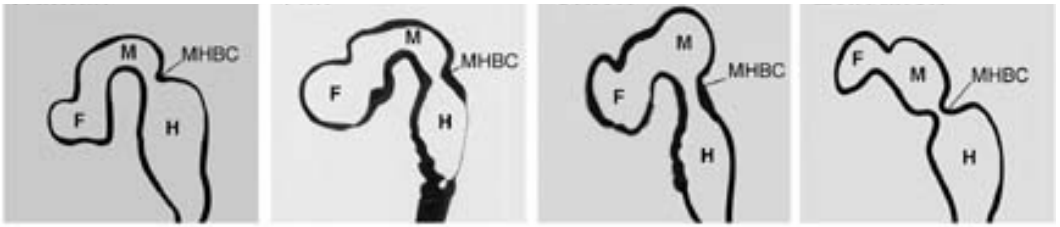
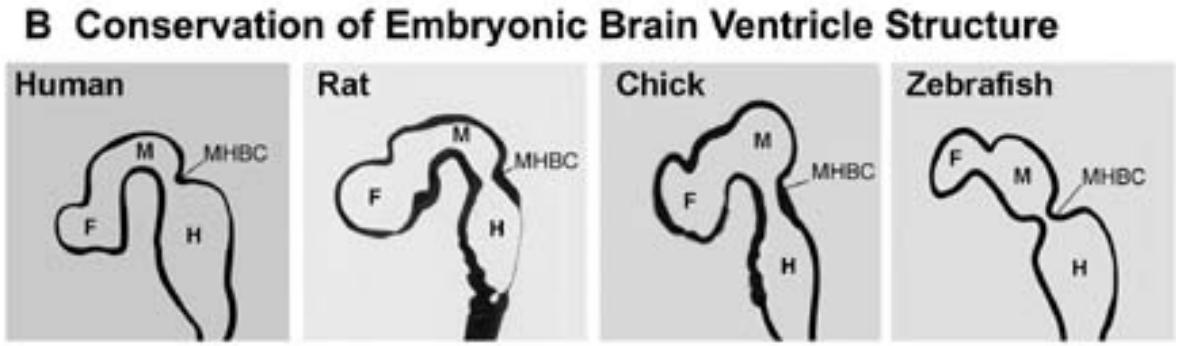
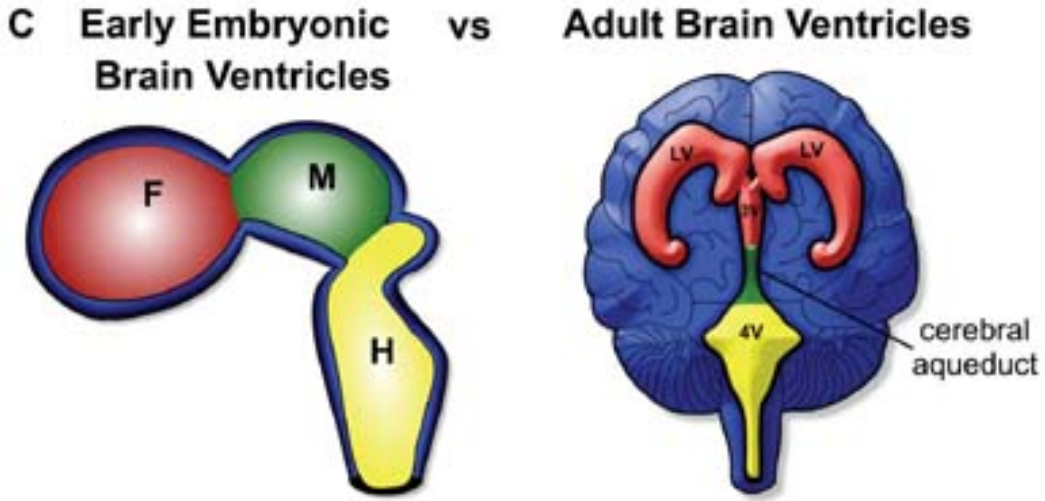




Human: 43 dpf
Rat:14 dpf
Chick: 2.5 dpf
Zebrafish: 24hpf



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

https://upload.wikimedia.org/wikipedia/commons/2/29/Hirnventrikel_mittelgroß.gif

Lowery and Sive BioEssay 2009

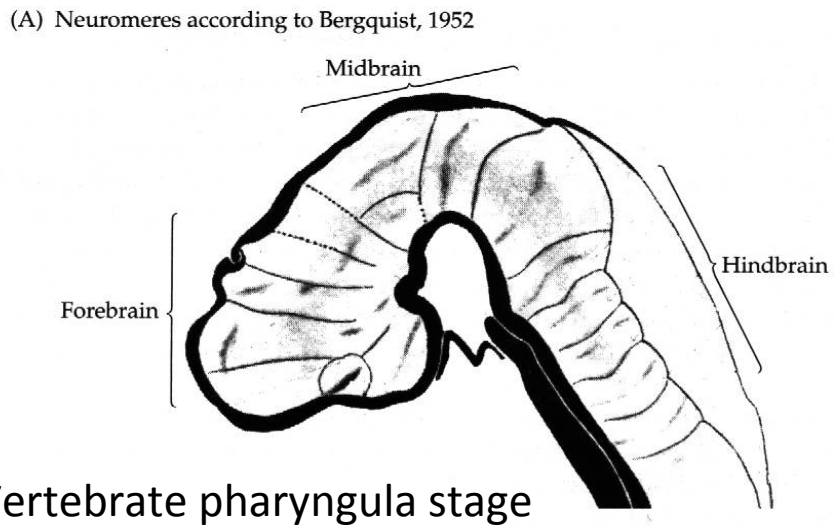
Comparative Neuroembryology

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.

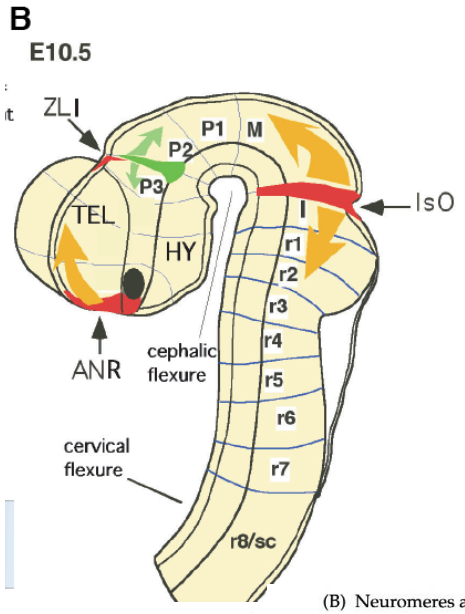


Vertebrate pharyngula stage

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****…*

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

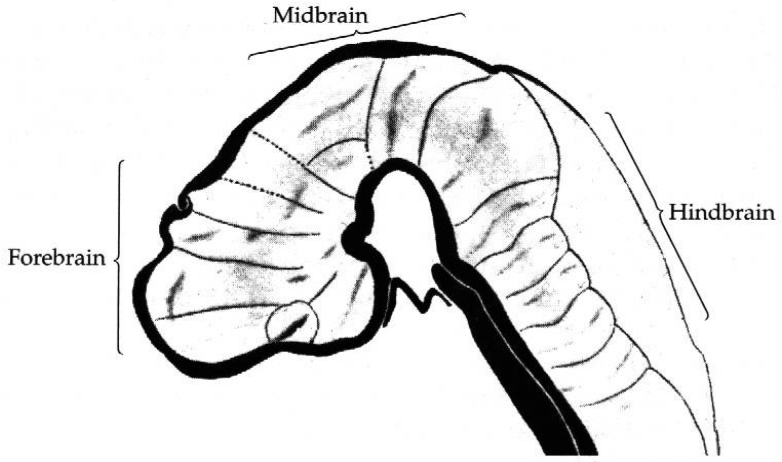
- Prosomeres
- Mesomeres
- Rhombomeres



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

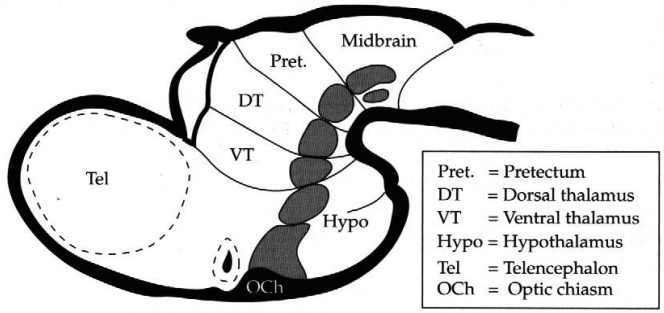


Figure 2.15 Versions of the "Neuromeric Model" Comparative neuroembryologists in Sweden during the first half of the twentieth century proposed that embryonic vertebrate brains are divisible into numerous transverse segments, or neuromeres, which are separated from one another by external grooves. This 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

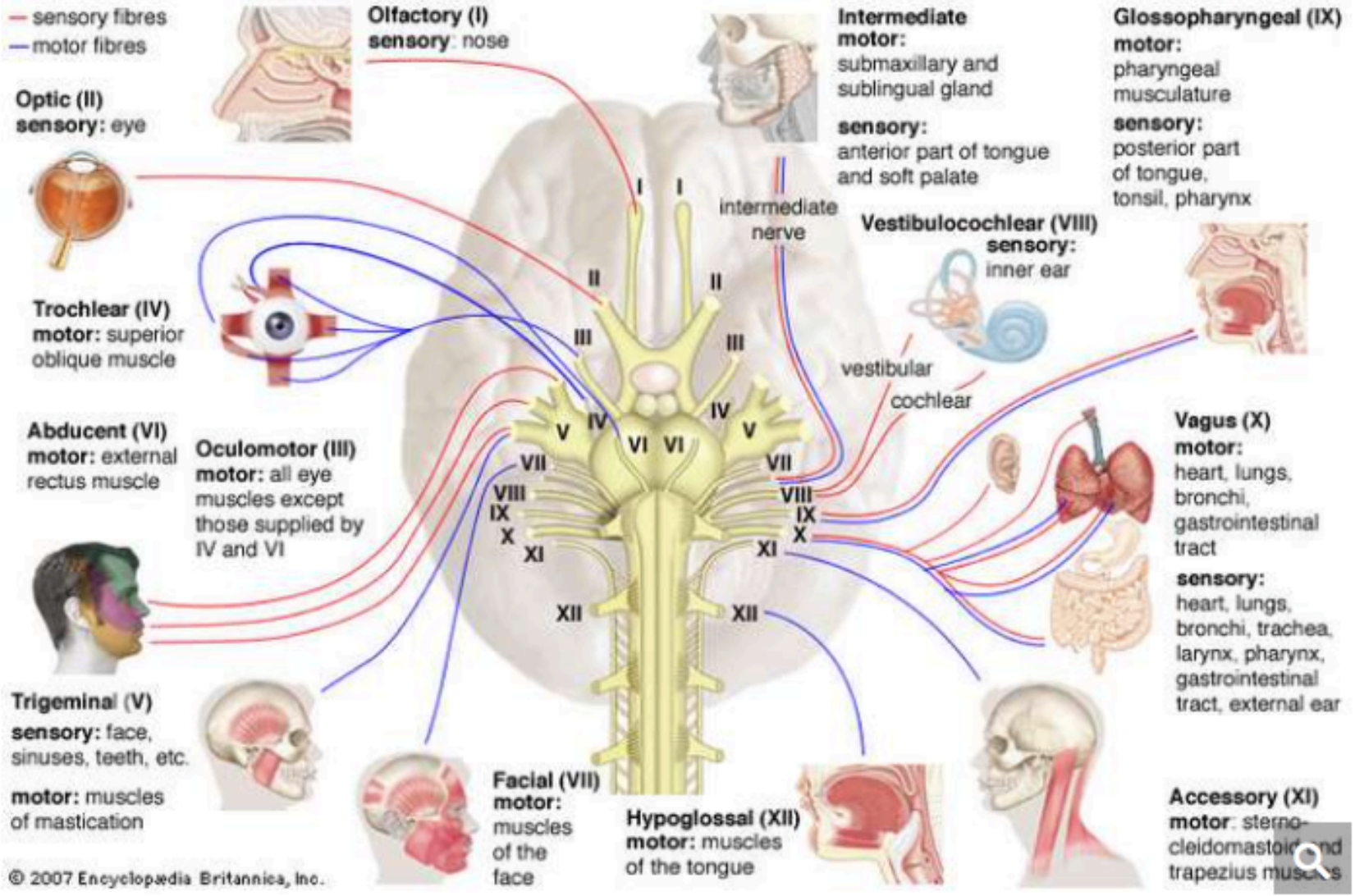
Precursor cells become regionalized => their potential is based on their **spatial position**

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

Genetic programs acting along the rostrocaudal axis provide an important means through which neuronal classes establish subtype identities

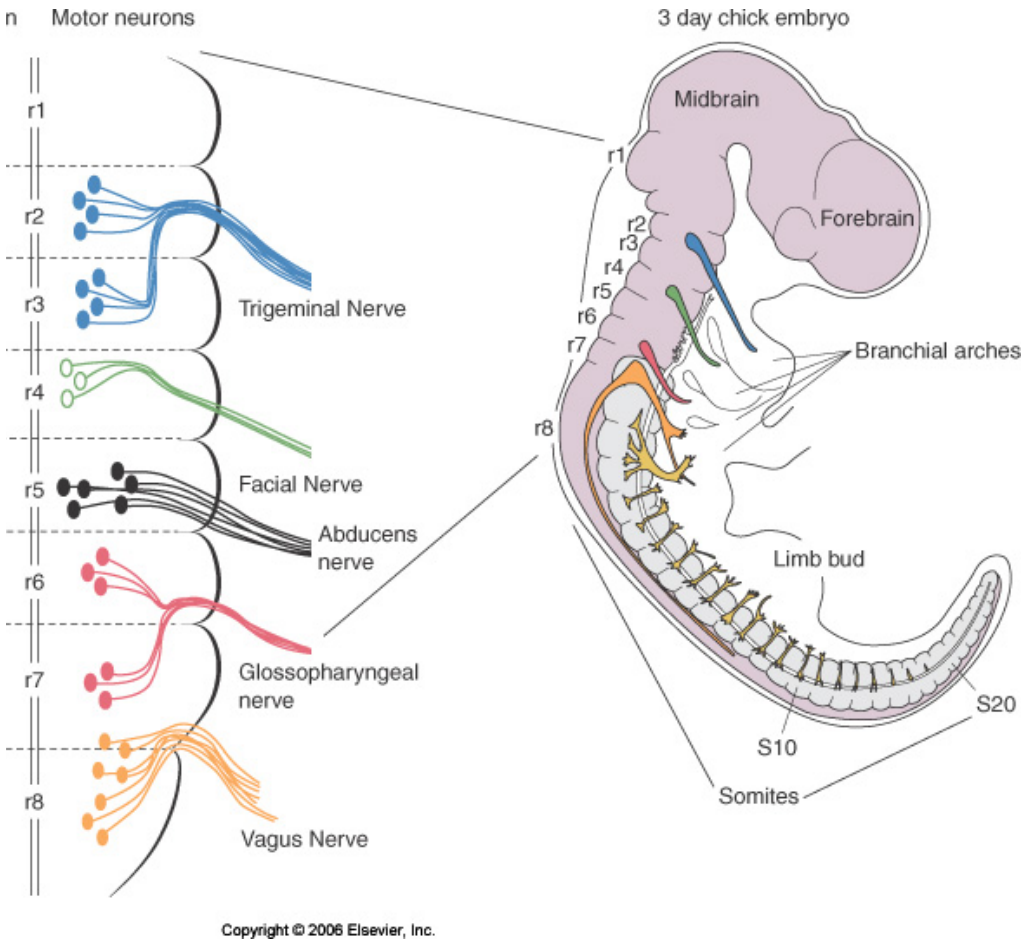
→ The example of the hindbrain: one of the clearest area of segmentation in the brain

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



Cranial nerves

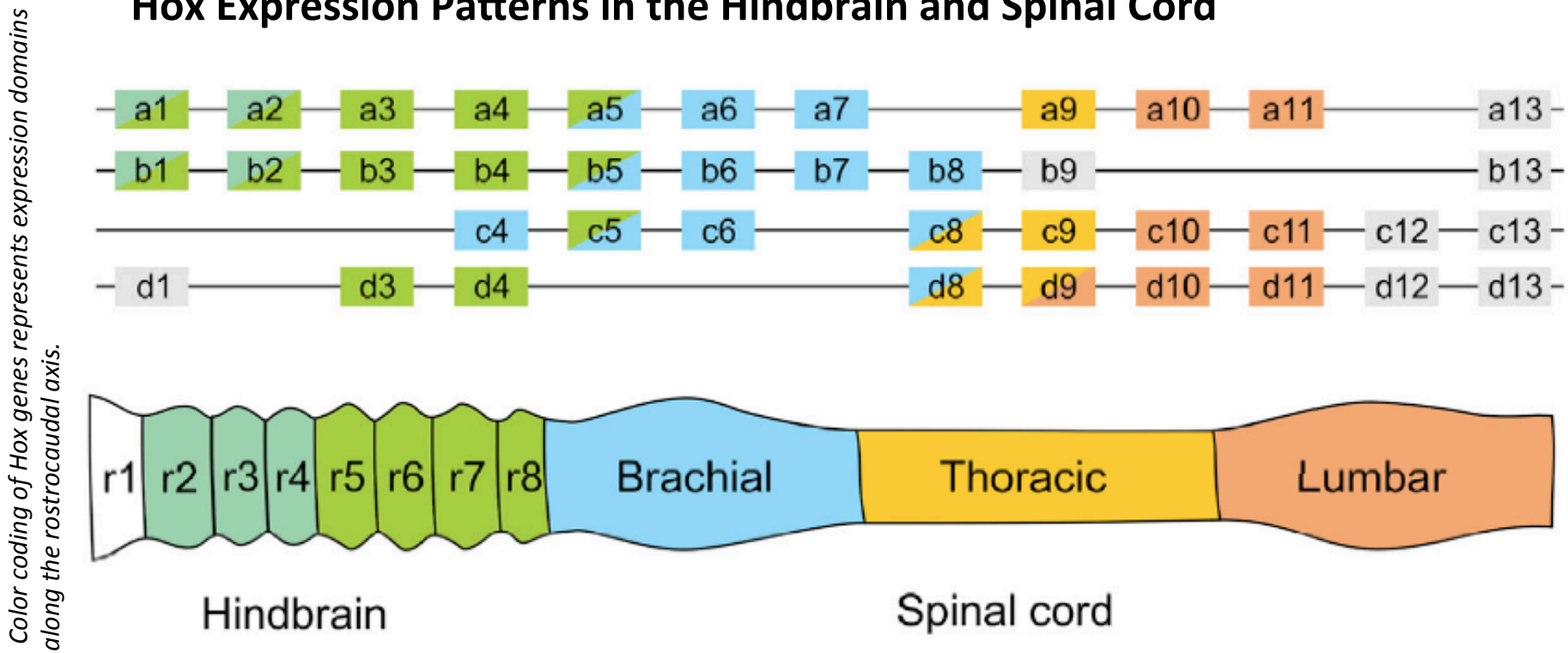
Rhombomers in the developing hindbrain



- Hindbrain rhombomeres are transient, serially homologous structures separated by distinct boundaries
- no physical barriers exist between rhombomeres, differential cell adhesion properties (two-segment periodicity) prevent intermixing of cells between compartments
- 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?

Hox Expression Patterns in the Hindbrain and Spinal Cord



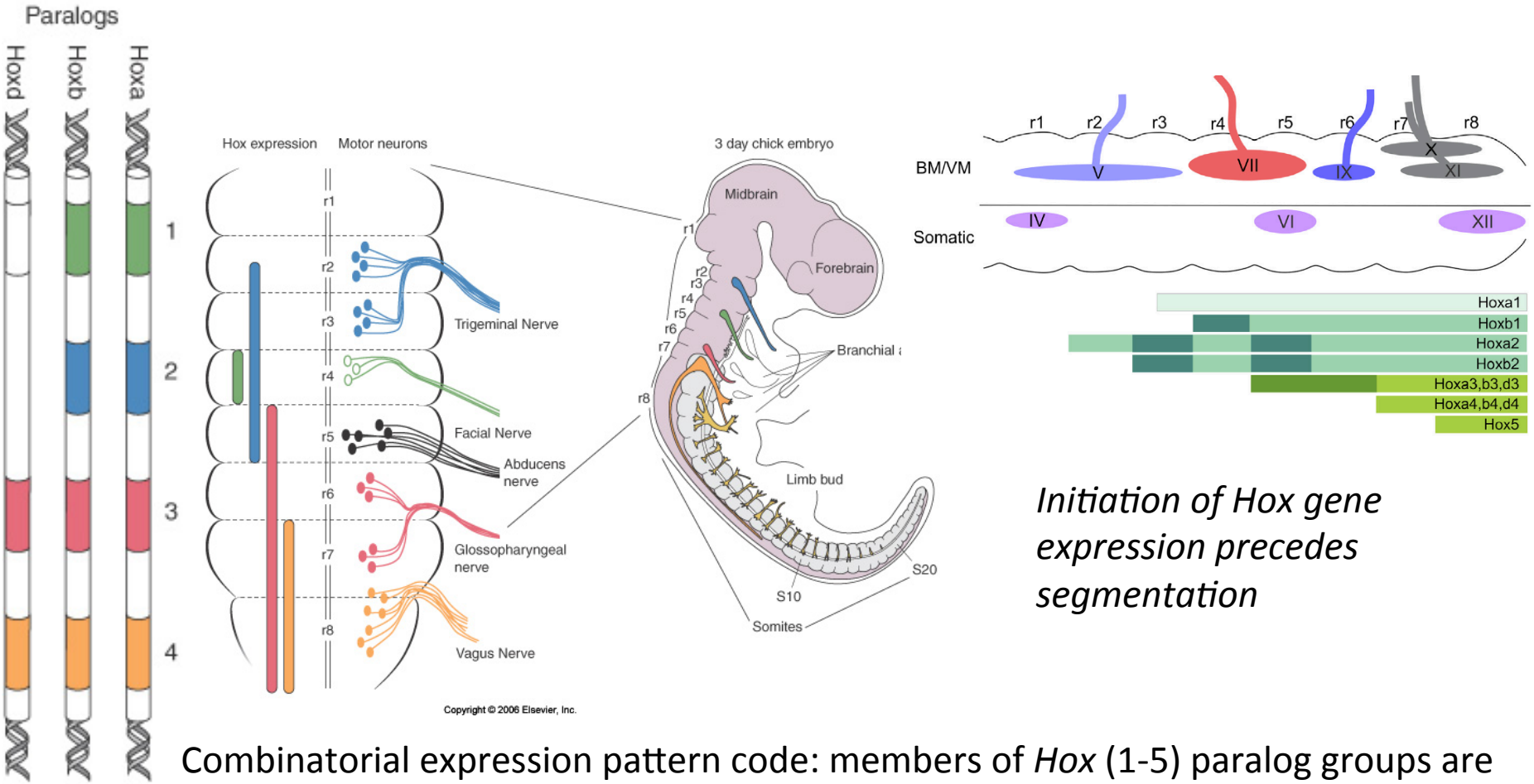
Hox genes are found in all animal species and have conserved role in body patterning

In vertebrates, 39 Hox genes are distributed across four clusters.

Each Hox gene is expressed in discrete rostrocaudal domains within the hindbrain and spinal cord.

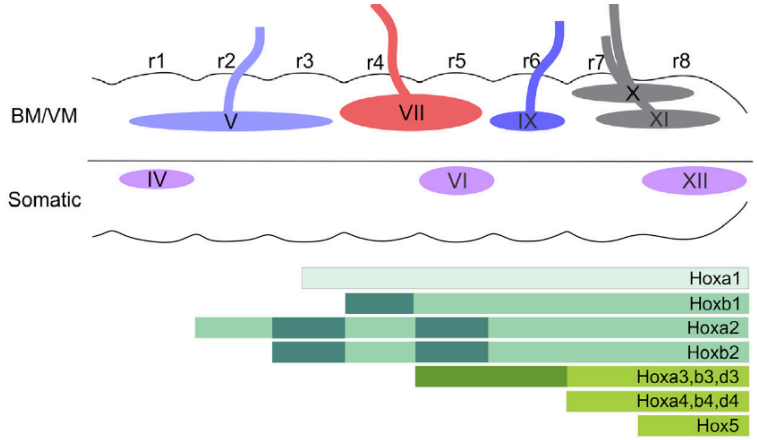
Hox gene expression in the hindbrain and spinal cord is **spatially** and **temporally** dynamic

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



Initiation of Hox gene expression precedes segmentation

Combinatorial expression pattern code: members of *Hox* (1-5) paralog groups 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**



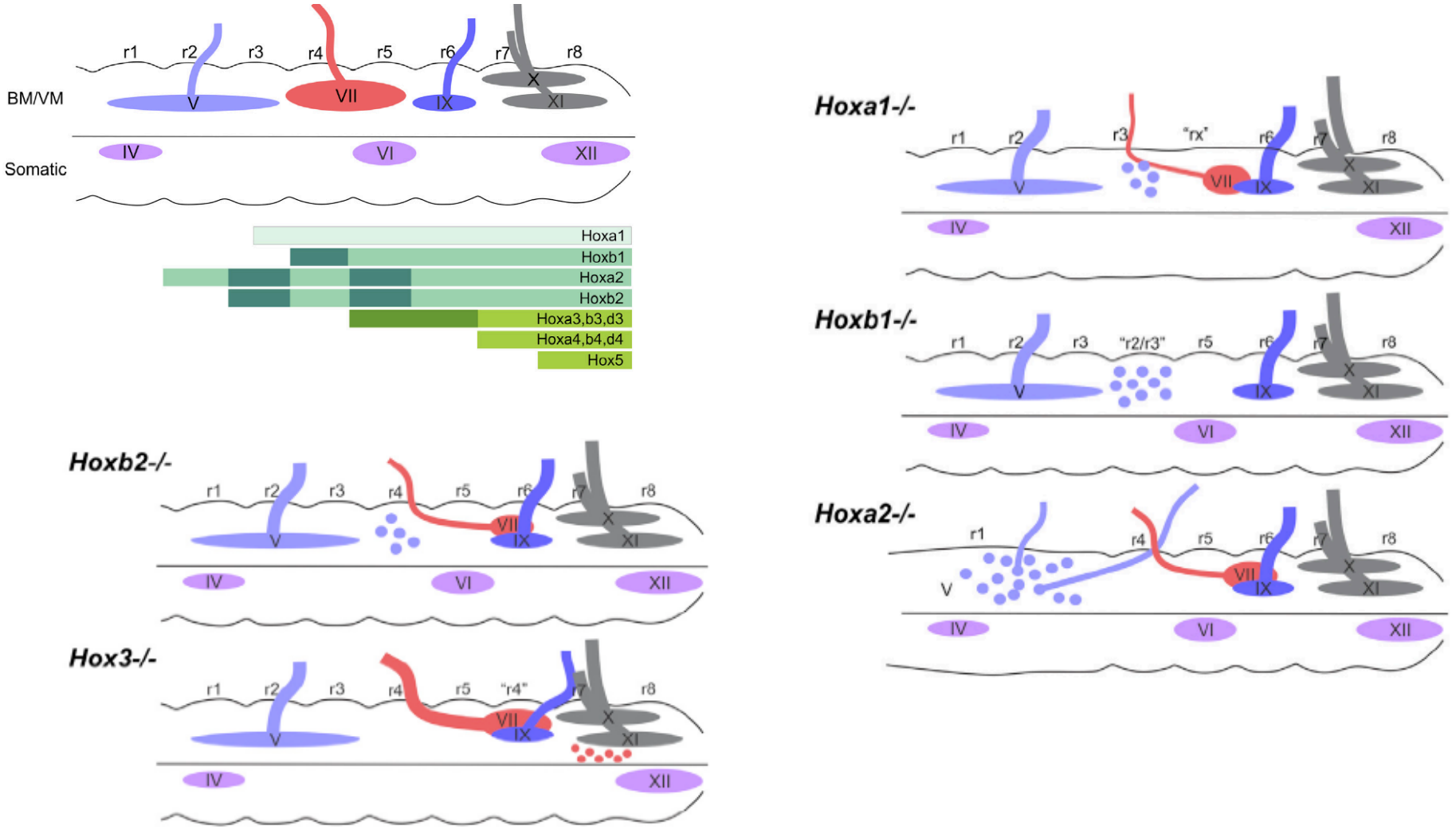
Motor nuclei (MN):
Branchiomotor (bm) → project their axons dorsally-to innervate parasympathetic ganglia
Visceral (vm) → project their axons dorsally - to innervate muscles in the pharyngeal arches
Somatic → located ventrally; innervate body muscles derived from paraxial or prechordal mesoderm

somatic MNs are derived from a progenitor domain expressing the transcription factor Olig2

bm and vm MNs are derived from Nkx2.2+ progenitors

All classes of hindbrain MNs require Hox genes for their development.

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



Mutations in Hox1–Hox3 genes result in misspecification, disorganization, and abnormal projections of hindbrain MNs

Development 118, 1063-1075 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

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

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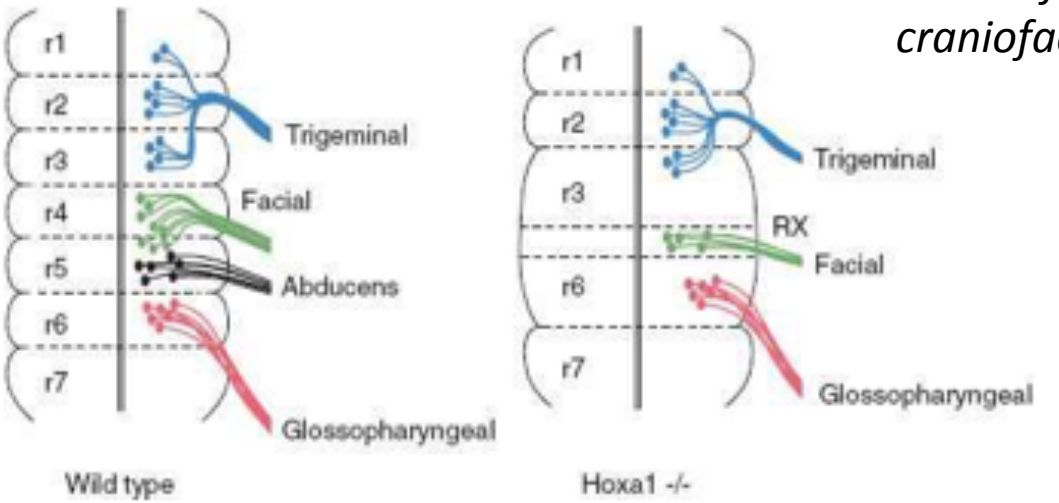
SUMMARY

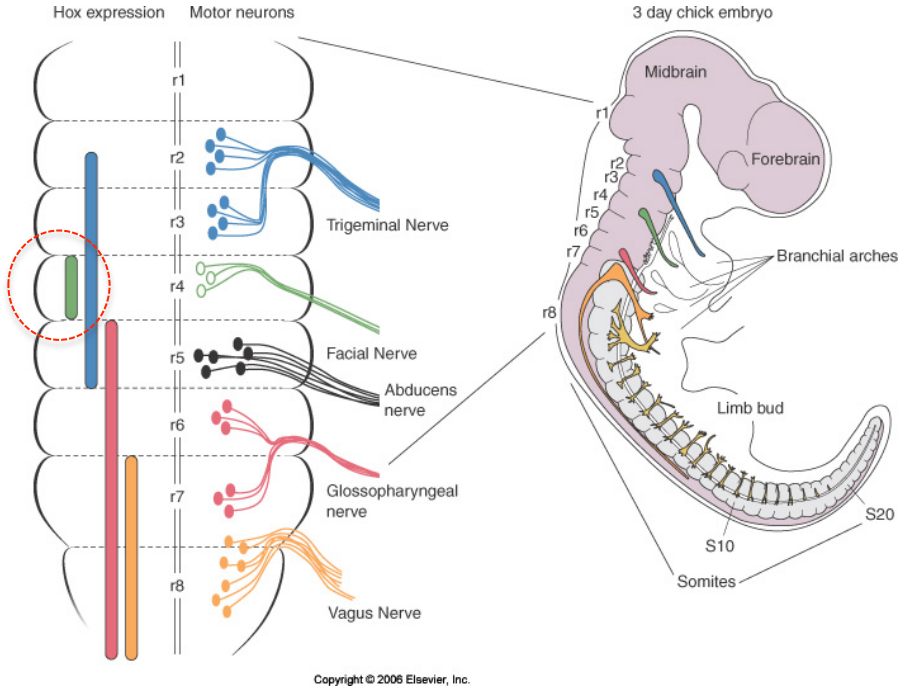
Targeted disruption of the murine *hox-A1* gene results in severe defects in the formation of the hindbrain and associated cranial ganglia and nerves. Carbocyanine dye injections were used to trace afferent and efferent projections to and from the hindbrain in *hox-A1*$^{-}$/*hox-A1*$^{-}$ mutant mice. 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

Defects in hindbrain and craniofacial development ***in Hoxa1-/-***



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



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

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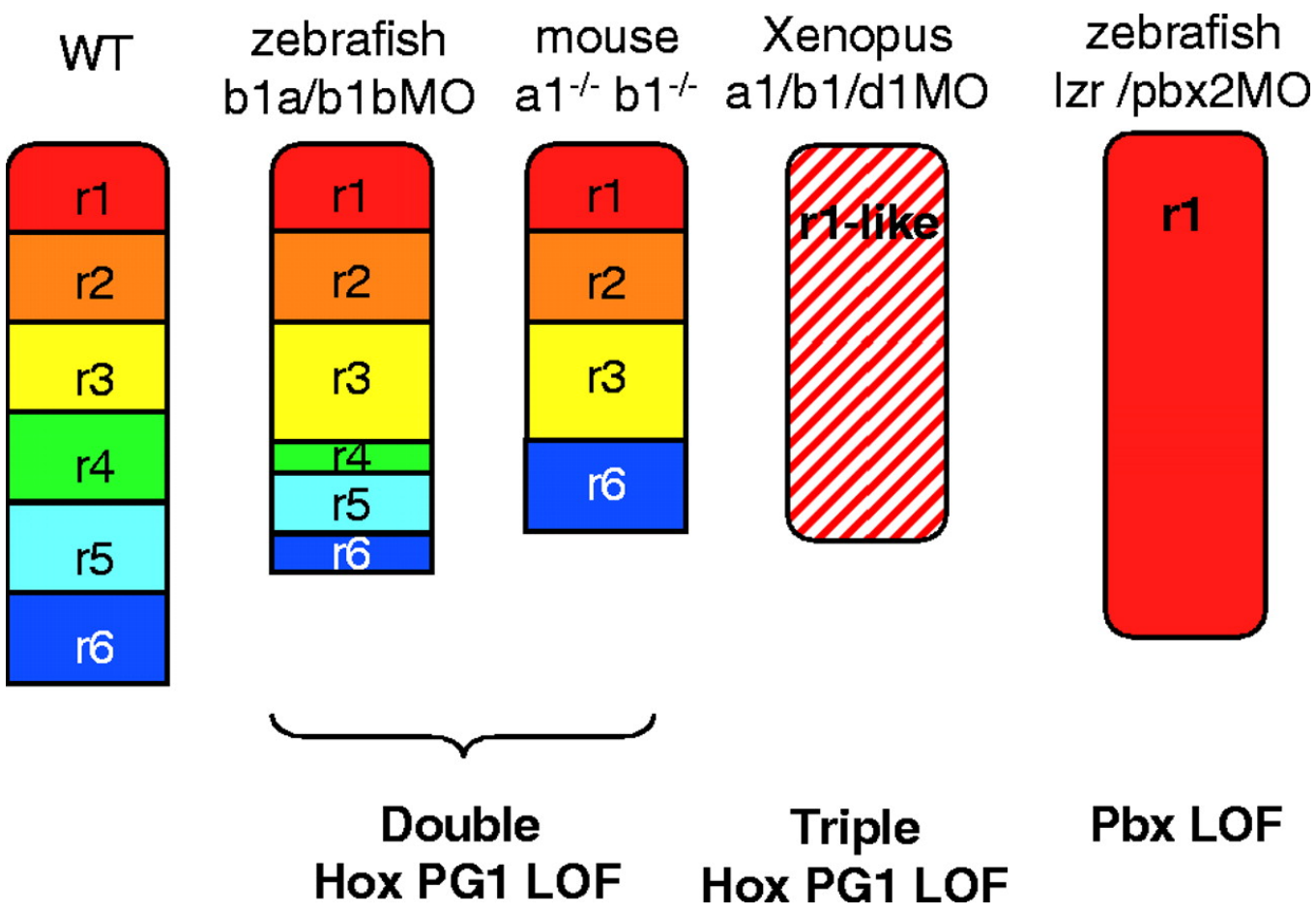
Development

Summary

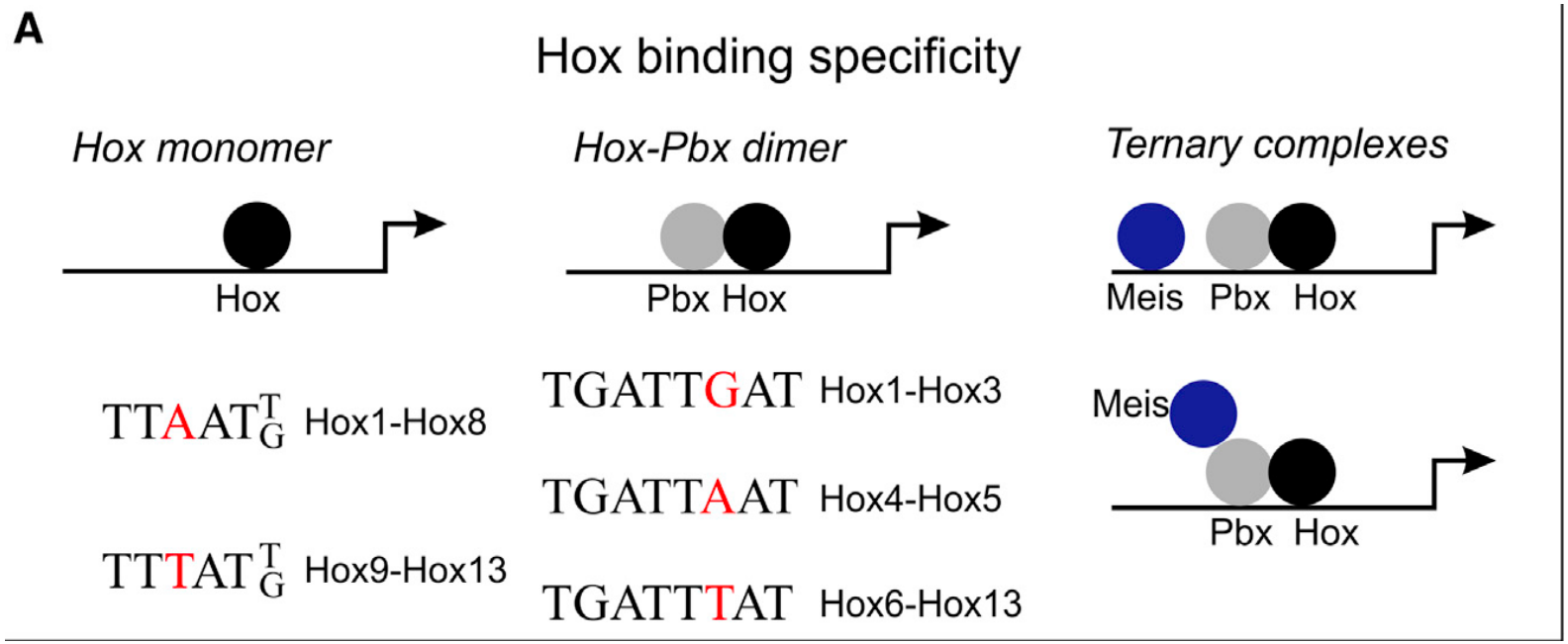
The Hox paralogous group 1 (PG1) genes are the first and initially most anterior Hox genes expressed in the embryo. In *Xenopus*, the three PG1 genes, *Hoxa1*, *Hoxb1* and *Hoxd1*, are expressed in a widely overlapping domain, which includes the region of the future hindbrain and its associated neural 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**



Hox proteins bind AT-rich hexamer sequences through homeodomains, which are conserved among Hox paralogs → low selectivity in their binding motif

Interaction with the TALE (three amino acid loop extension) class homeodomain proteins Pbx and Meis enhances binding specificity to target sequences and allow different Hox proteins to accomplish specific functions

Due to their role as cofactors, TALE gene mutants often exhibit phenotypes similar to Hox mutants in the CNS.

Dev Cell. 2002 Nov;3(5):723-33.

Eliminating zebrafish pbx proteins reveals a hindbrain ground state.

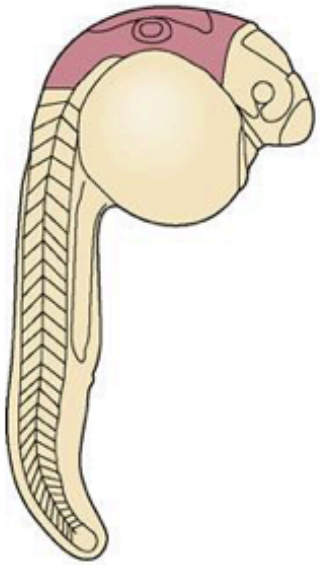
Waskiewicz AJ[1], Rikhof HA, Moens CB.

Author information

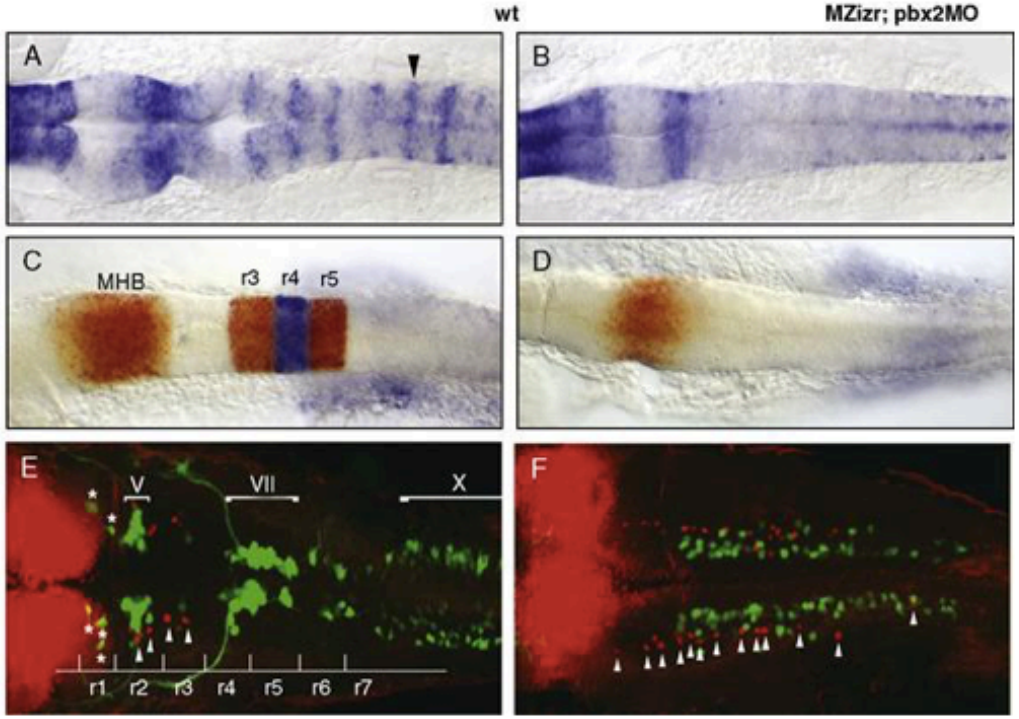
Abstract

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



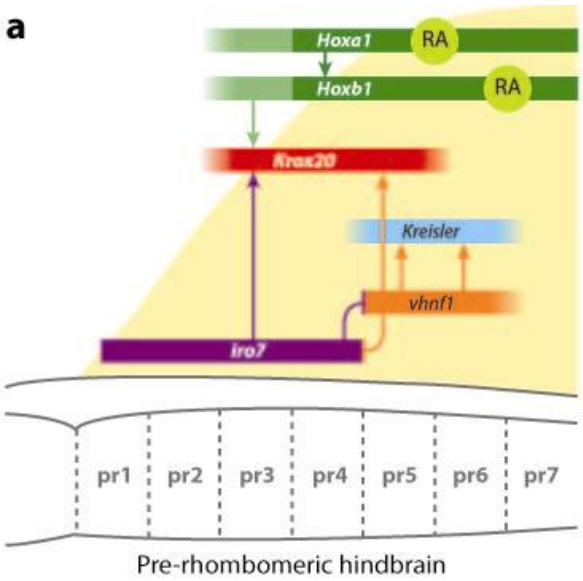
Gene expression

Neuronal types

→ hindbrain neuron identity is homogenized in embryos lacking Pbx function

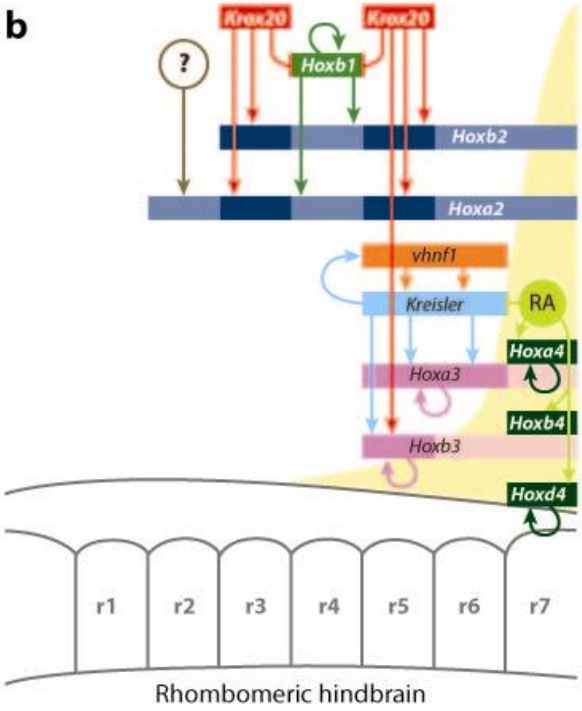
What control the patterned expression of Hox genes in vertebrates?

Network
prior to the appearance
of the rhombomeres



The yellow background
represents
the retinoic acid (RA)
gradient

Network
at the appearance
of the rhombomeres

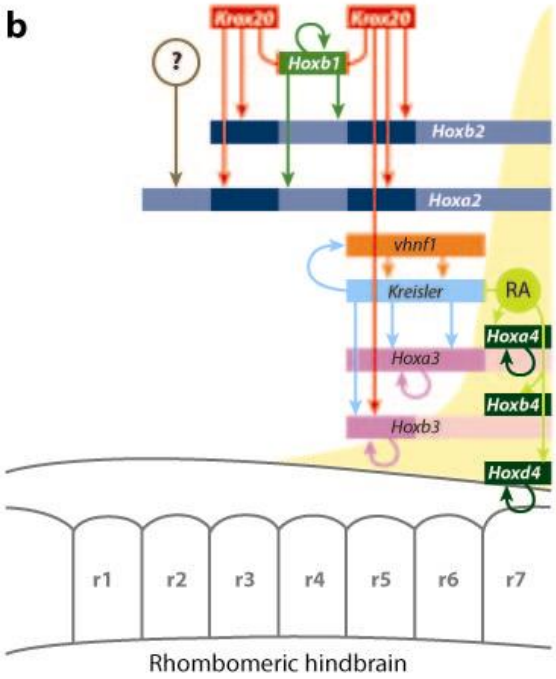


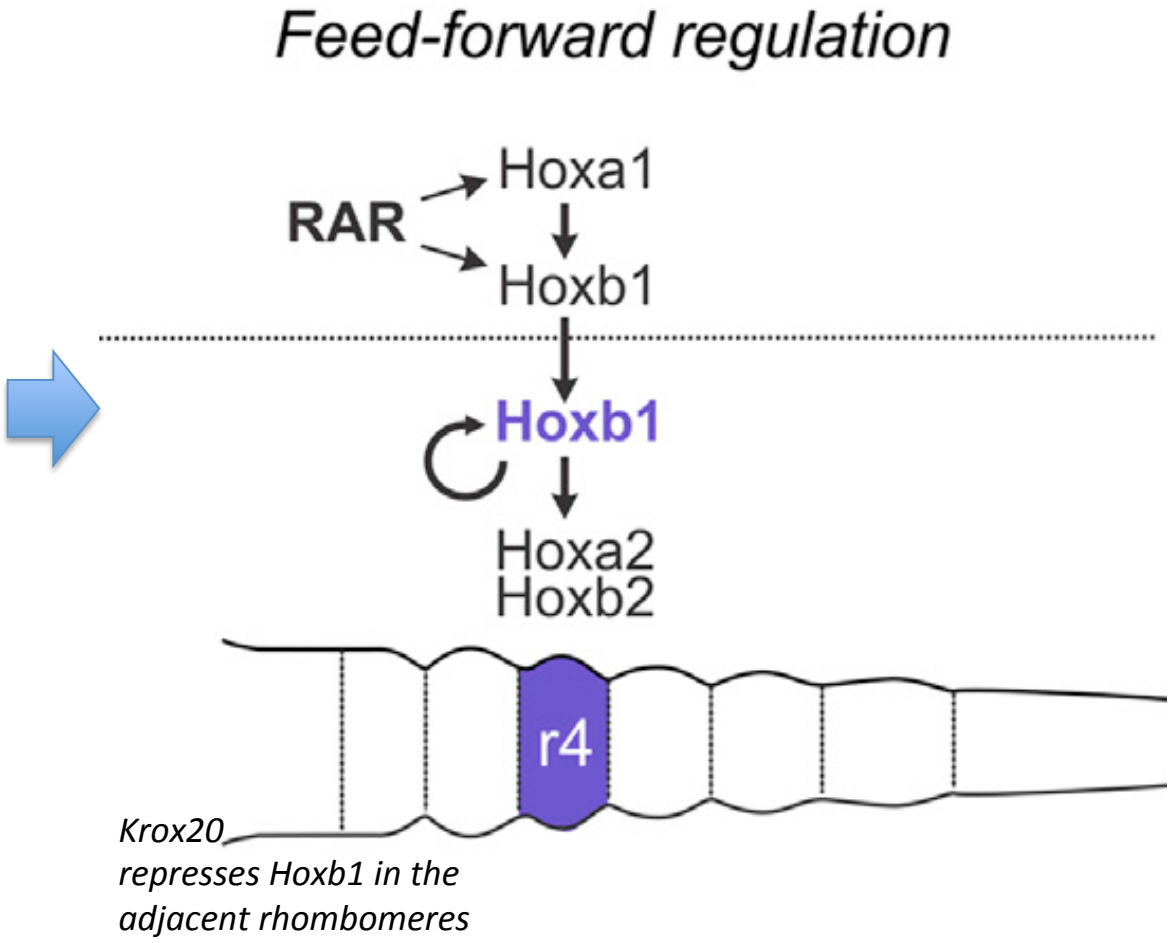
zebrafish

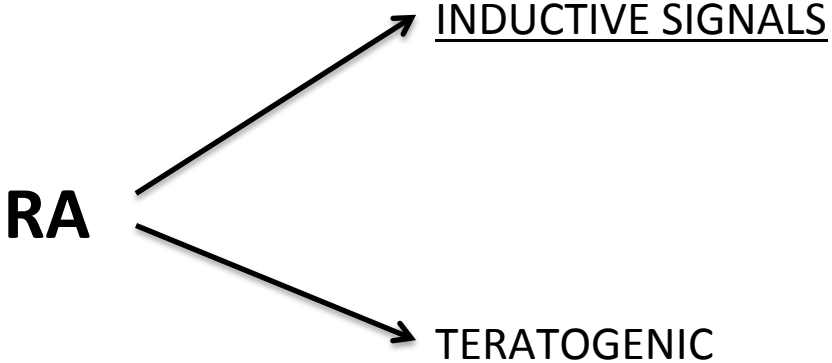
Alexander et al., 2009 Annu Rev Cell dev Biol

1) The initial inductive phase involves the sequential activation of genes within a Hox cluster and is mediated by **morphogens** acting in a graded manner along the rostrocaudal axis.

2) Refinement and maintenance of Hox patterns occurs at or near the time neurons become postmitotic through auto- and crossregulatory interactions between Hox proteins.





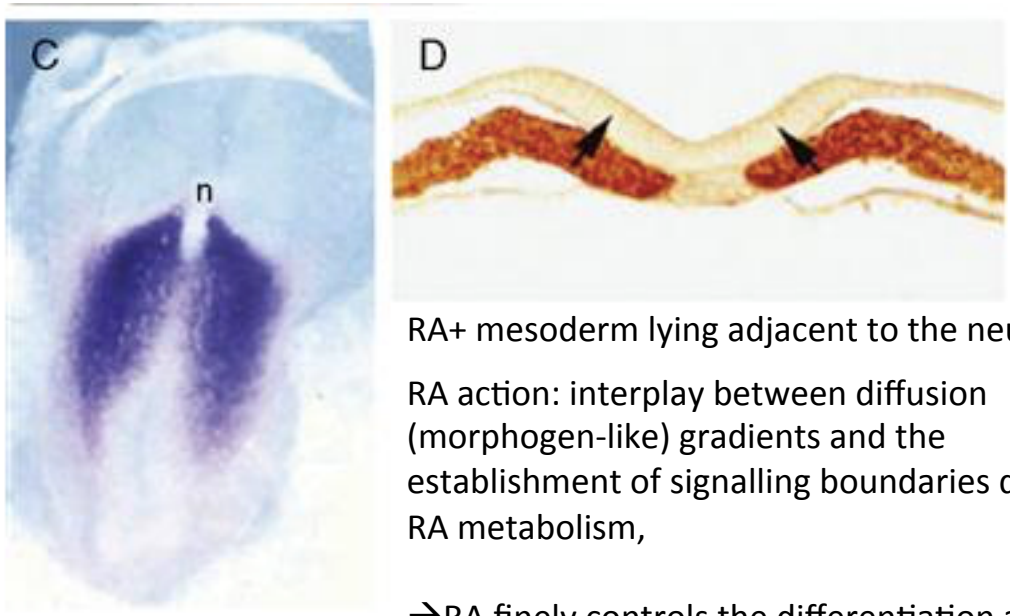


In vitro addition of RA to rapidly proliferating mouse ES cells, cultured in suspension as embryoid bodies, leads to the selective generation of neural progenitors with characteristics of radial glial cells found in the developing central nervous system

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

(Craniofacial and brain abnormalities)

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



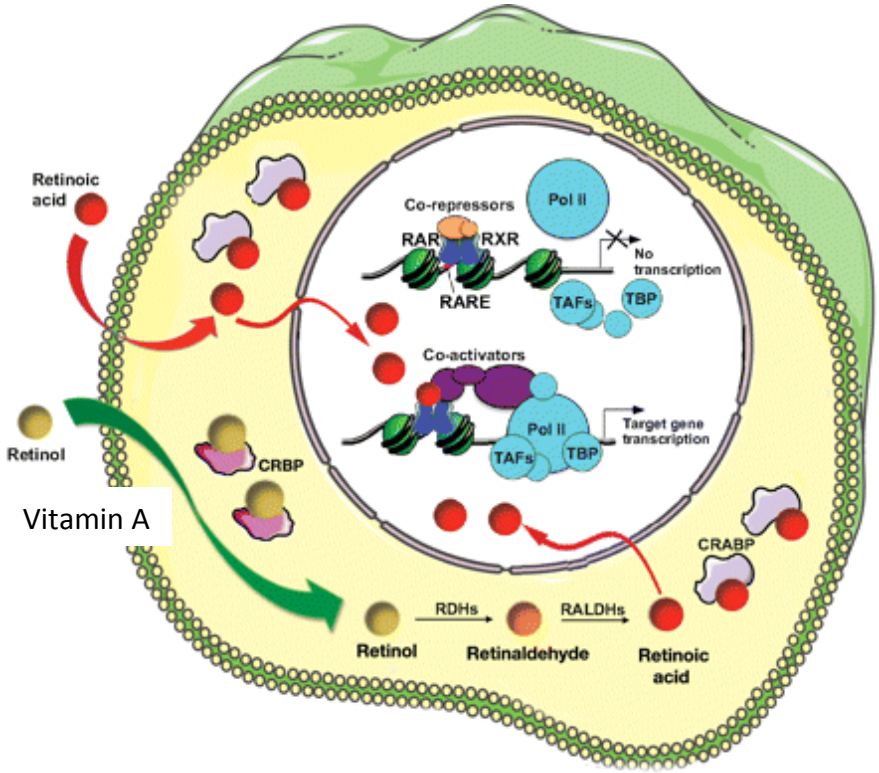
RA+ mesoderm lying adjacent to the neural tube

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



Rhinn et al., Development 2012

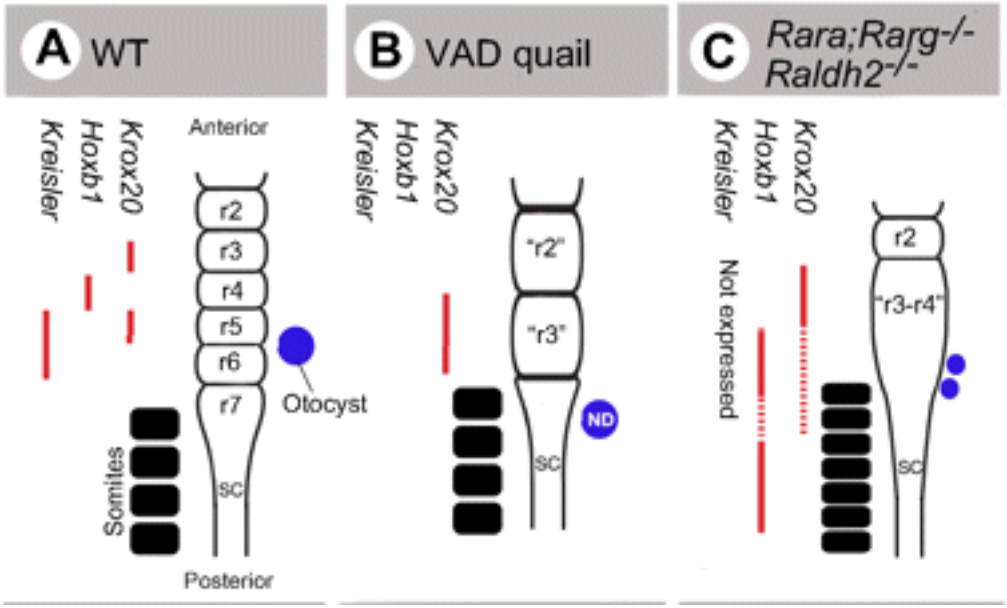
RDHs=retinol dehydrogenases
RALDHs=retinaldehyde dehydrogenases

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

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

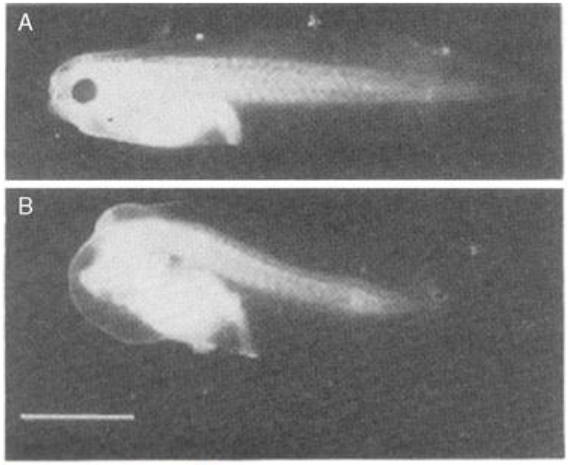


animal models **with endogenous deficiency in RA signalling**:

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

(C) Raldh2–/–, as well as Rara–/–;Rarg–/– signalling mutant **mice** (lacking RARα and RARγ);

alteration in posterior brain development



RA-treated Xenopus embryo

defects in anterior part of CNS

In vertebrates Hox gene expression is confined to the hinbrain and spinal cord BUT 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 Emx, Dlx and Pax…)