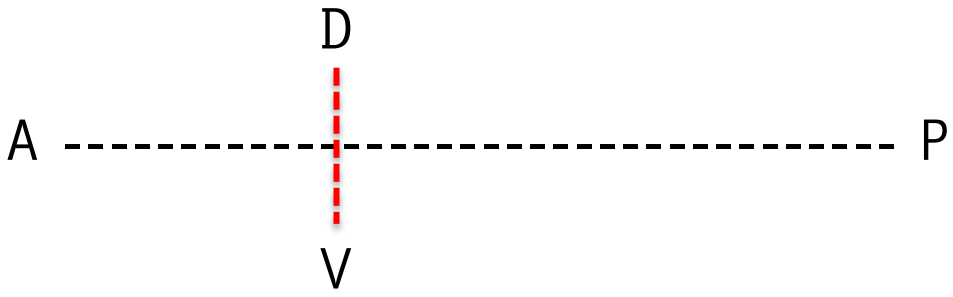
Patterning of the neural tube

Patterning of the neural tube



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 the organizer at different times during gastrulation results in induction of different parts of the neuraxis

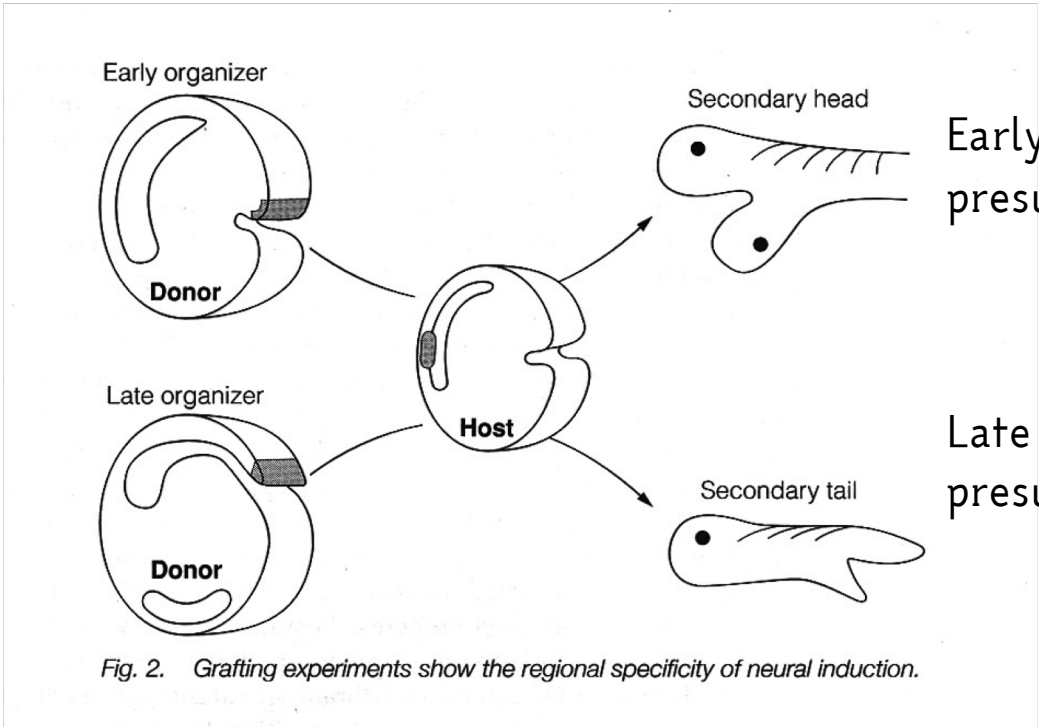
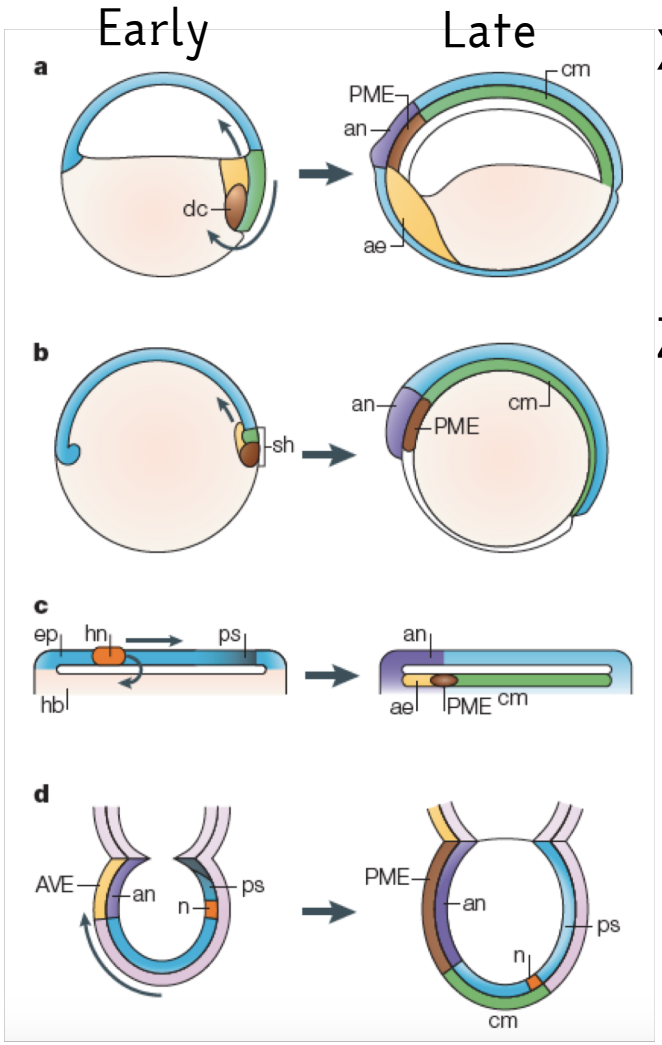


Fig. 2. Grafting experiments show the regional specificity of neural induction.

Early organizer: early gastrula lip
presumptive prechordal mesoderm → **head organizer**

Late organizer: late gastrula lip
presumptive chordal mesoderm → **tail organizer**

Gastrula



Xenopus

Zebrafish

Chick

Mouse

ae = anterior endoderm
PME = prechordal mesoderm
cm = chordal mesoderm
an = anterior neural plate
AVE = anterior visceral endoderm

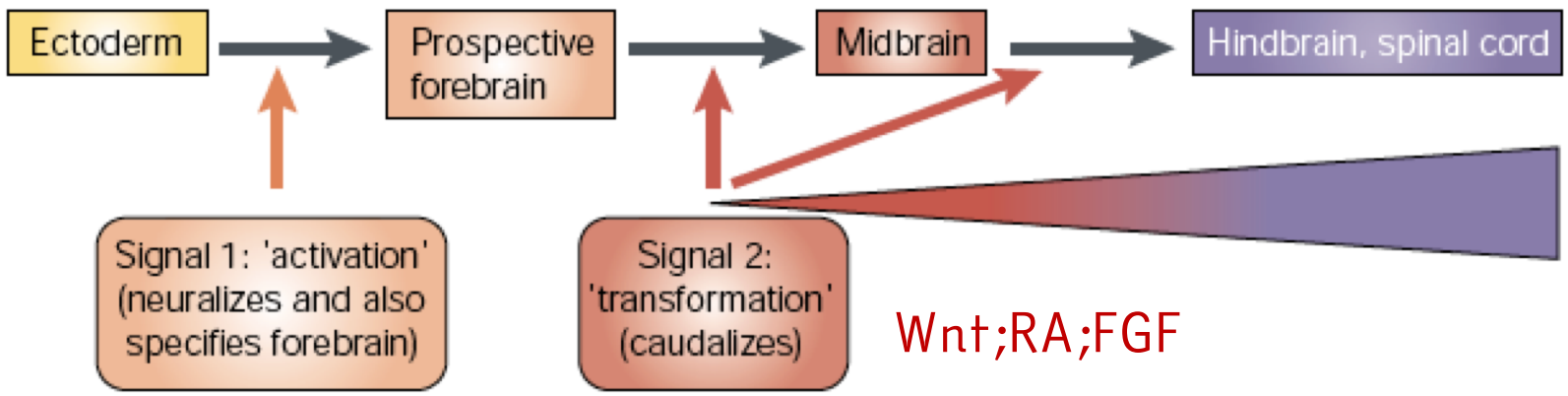
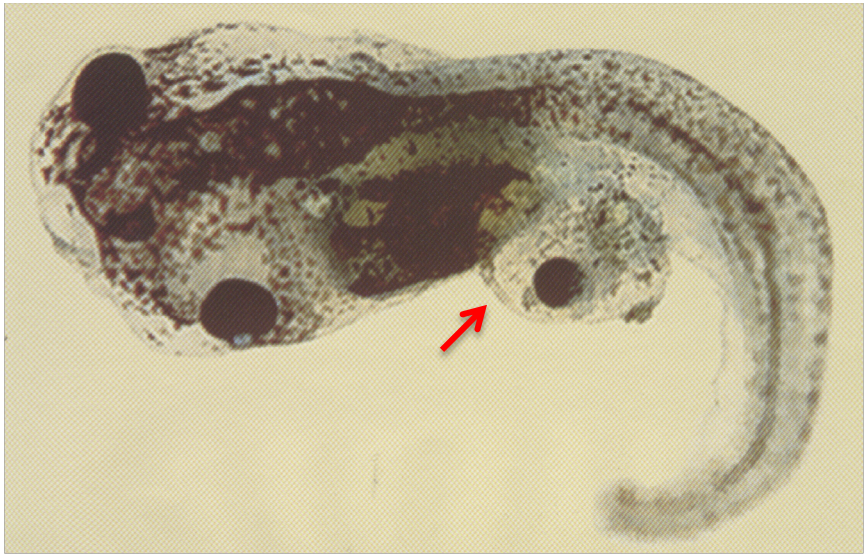


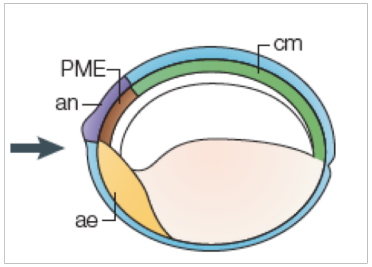
Figure 3 | **Nieuwkoop's activation–transformation model**[48]. Induction of the nervous system is proposed to occur in two steps: initial induction of forebrain, and subsequent caudalization of part of it. According to this model, the organizer emits both the activating and the transforming signals.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

Cerberus

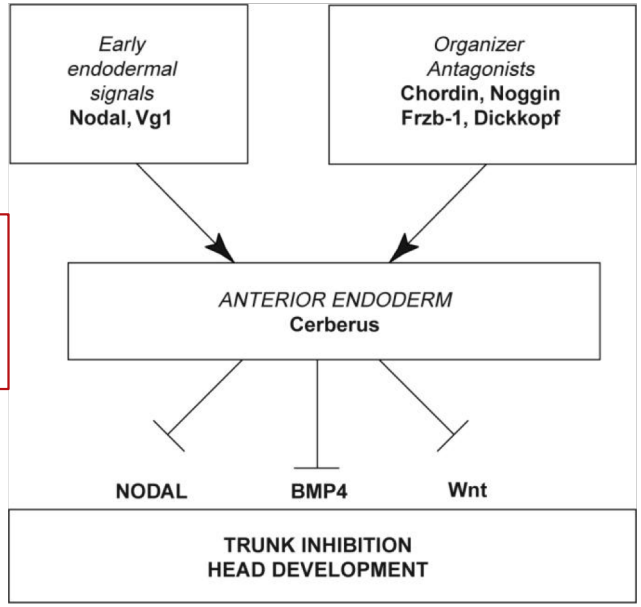
expressed in
- anterior primitive endoderm
- pre-chordal mesoderm



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



multivalent growth-factor antagonist

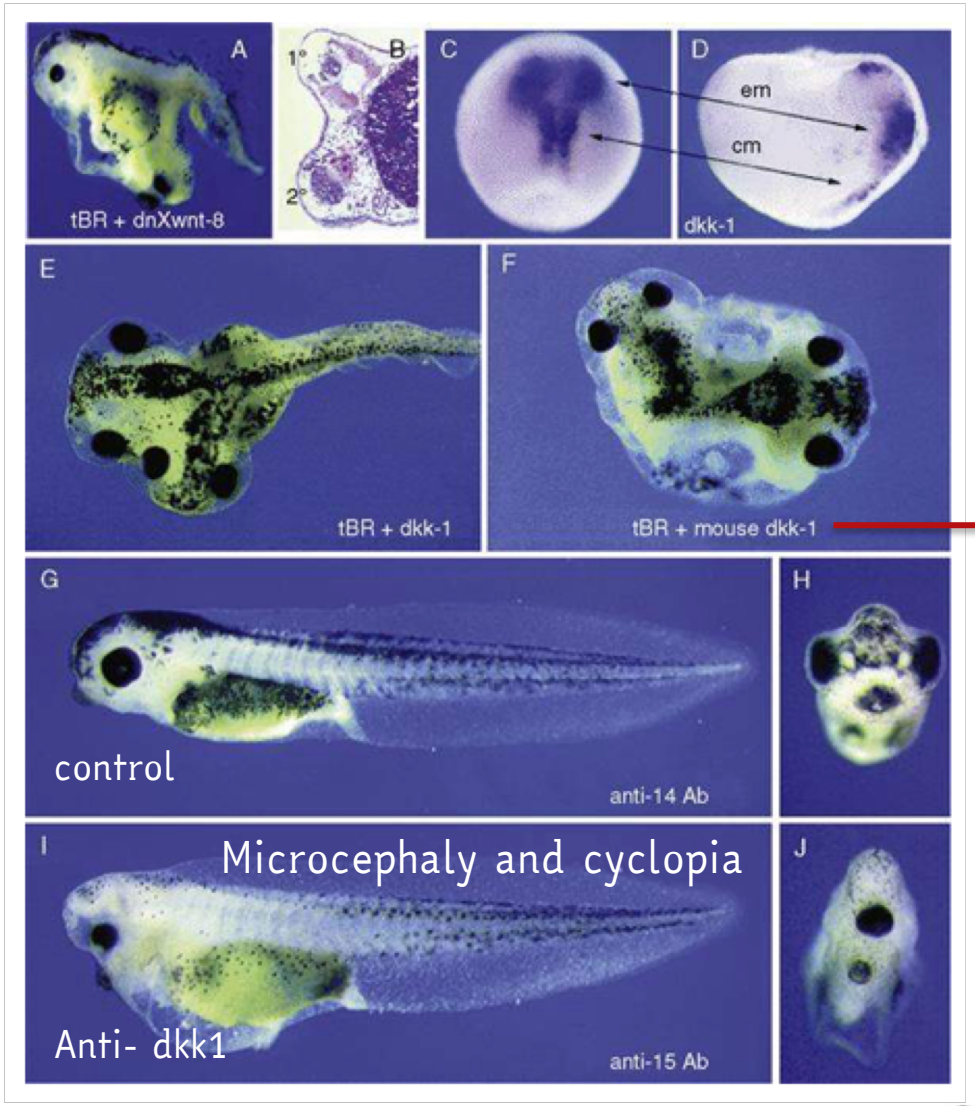


Dickkopf

tBR = truncated BMP receptor
dnXwnt-8 = dominant negative Wnt8
(A,E,F Injection of a four cell Xenopus embryo)

Em = endomesoderm
Cm = chordamesoderm

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



high conservations among evolution

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Dickkopf

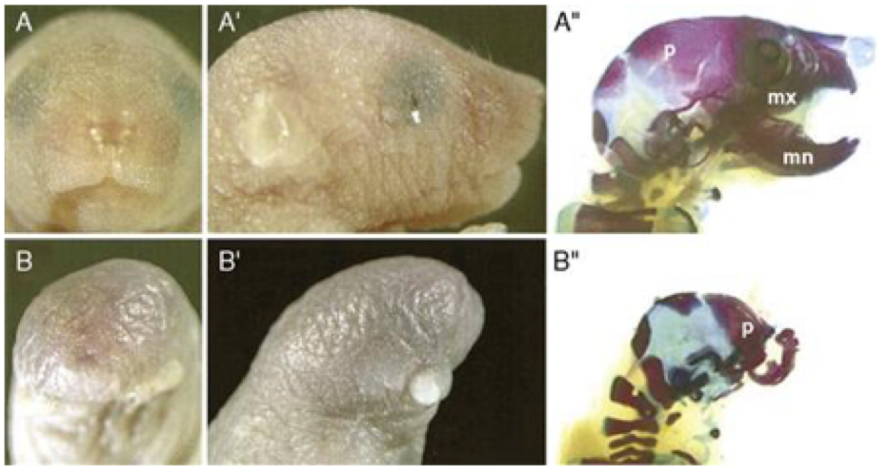


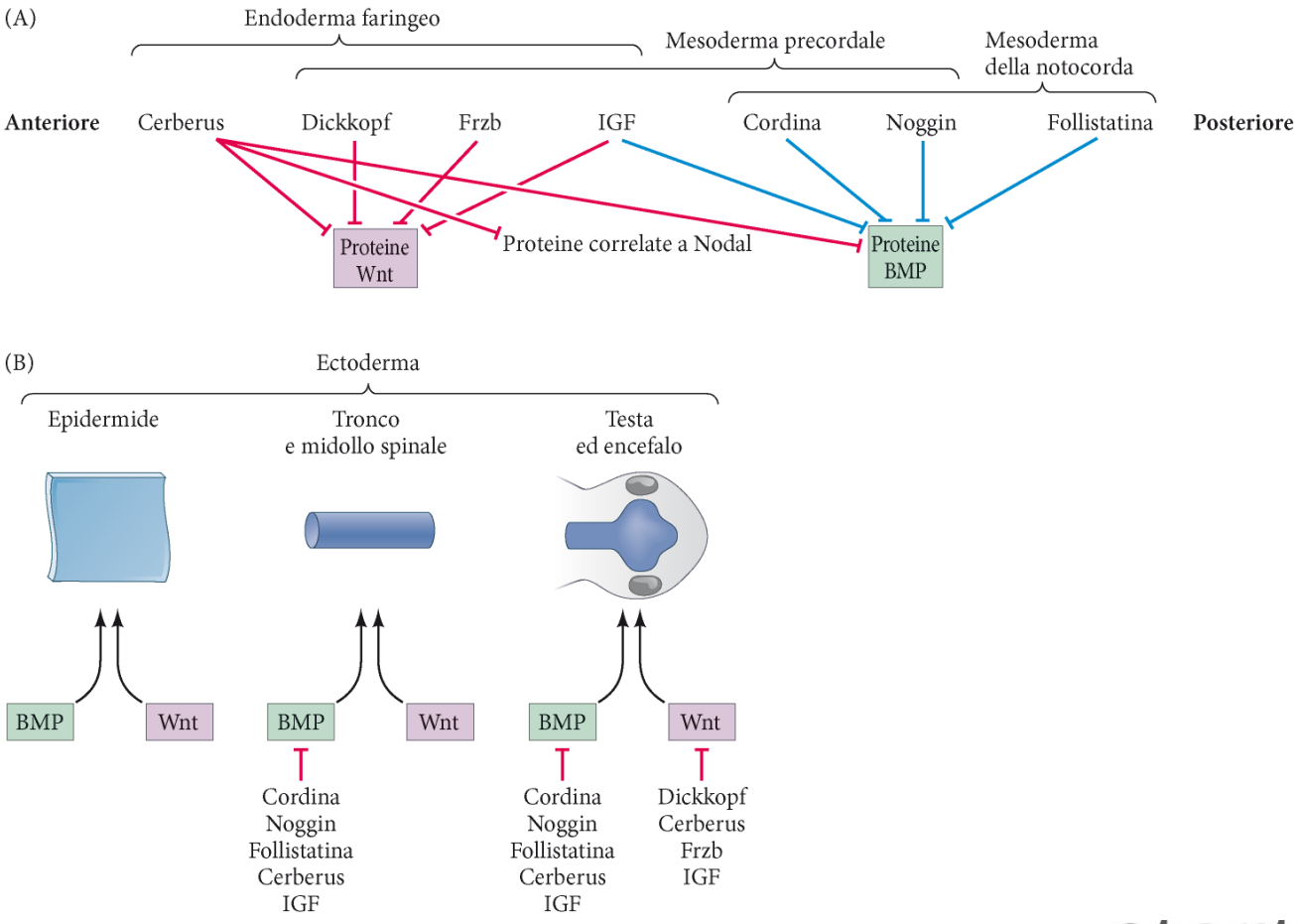
Fig. 2.10 *Dkk1* and *noggin* cooperate in head induction. Mice in which one allele for the genes for both *dkk1* and *nog* have been deleted have severe head defects. Frontal (*A*,*B*) and lateral (*A′*,*B′*) views of wild-type (*A*,*A′*) and mutant (*B*,*B′*), newborn animals. Lateral view of skeletal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

Dkk KOs lack head and brain structures anterior to hindbrain

Similar phenotype in Dkk+/-Noggin+/- mutants

Sinergy!

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

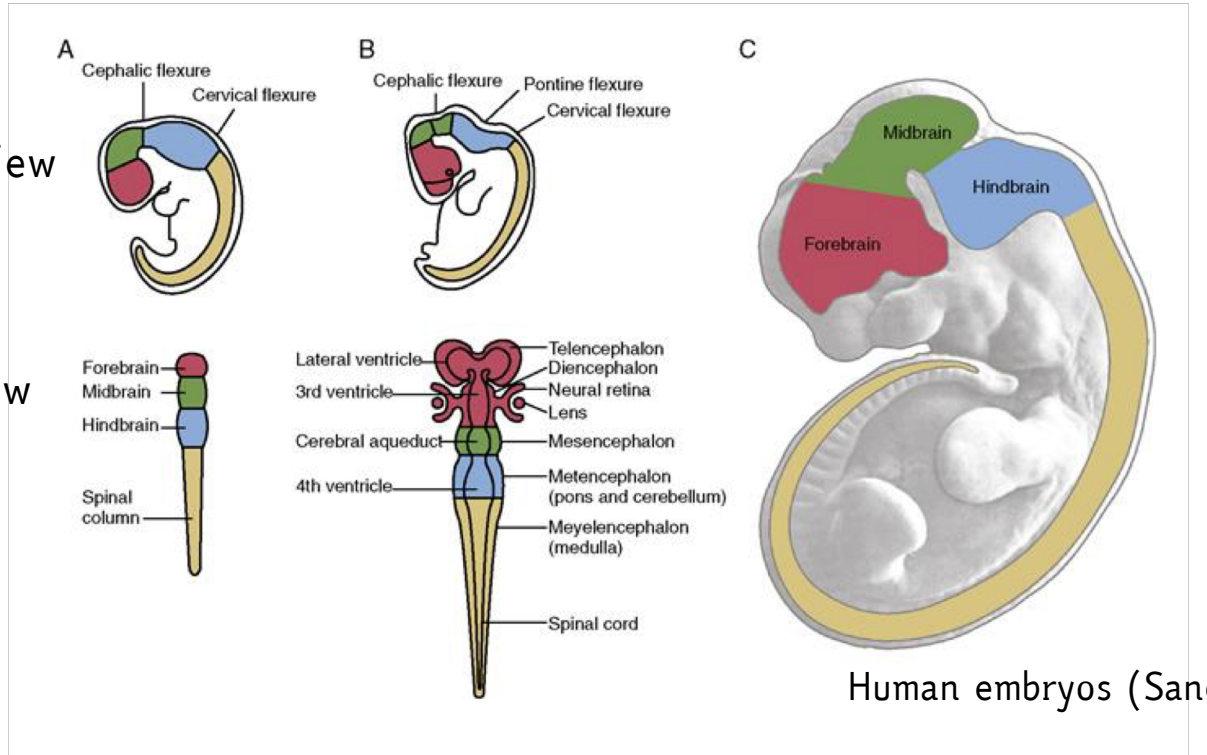


The nervous system is regionally specified during development

time →

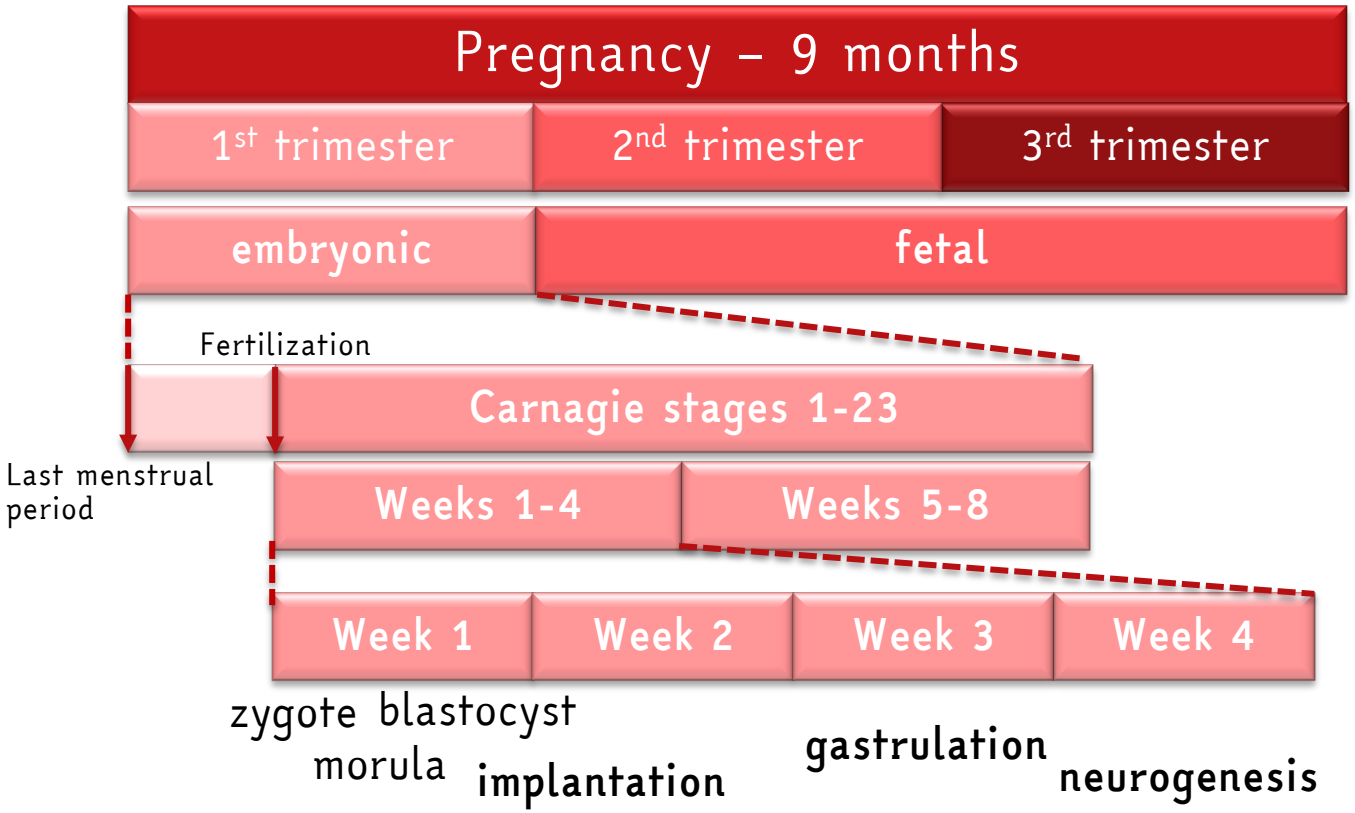
Lateral view

Dorsal view



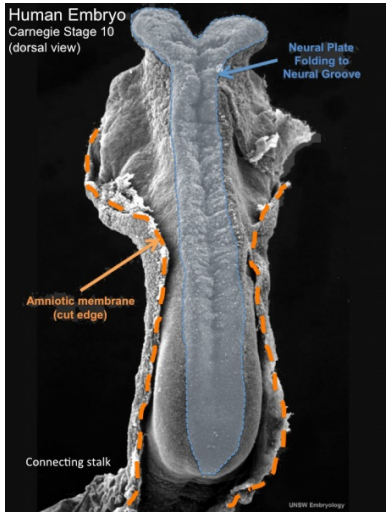
Human embryos (Sanes)

Human development Timeline

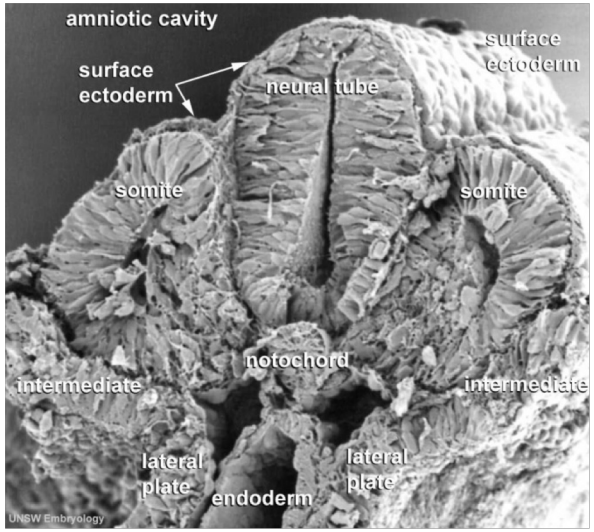


Human

Stage 8 – week 3



Stage 10 – week 3

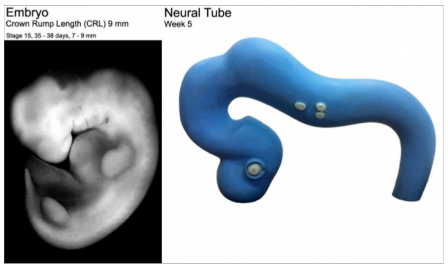


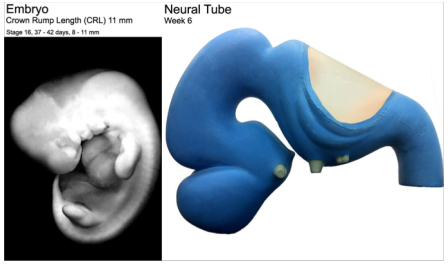
Stage 11 – week 4

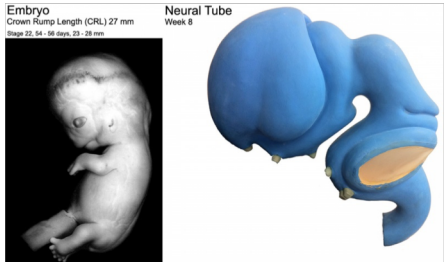
SEM

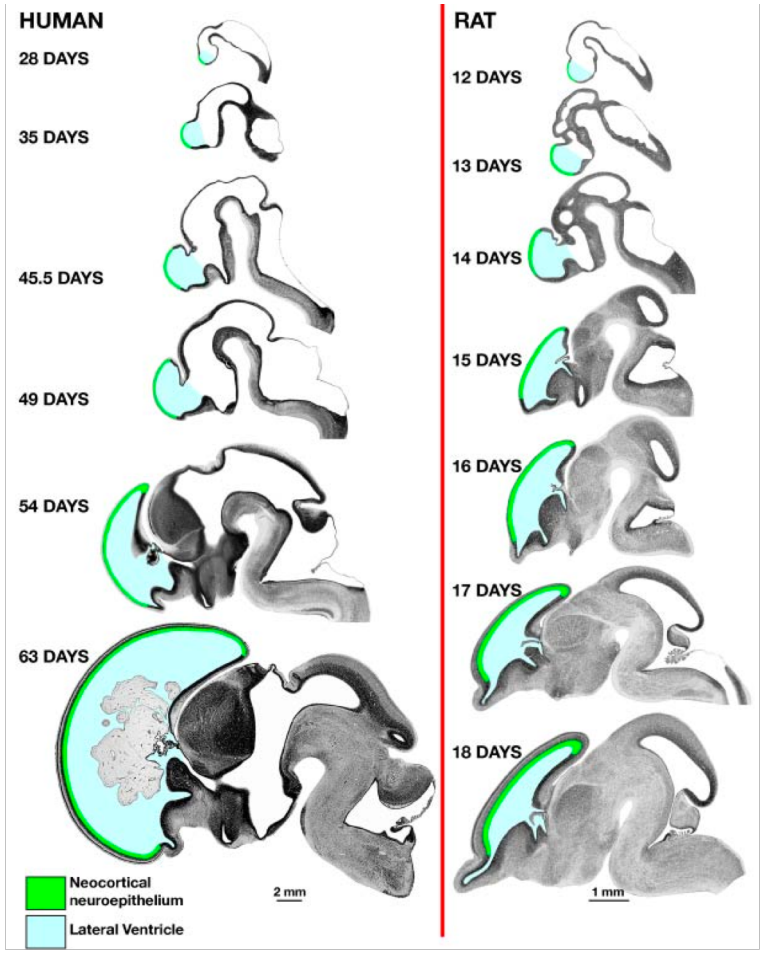
Time line – comparison based on Carnagies stages

Species	Stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Human	Days	1	2-3	4-5	5-6	7-12	13-15	15-17	17-19	20	22	24	28	30	33	36	40	42	44	48	52	54	55	58
Mouse	Days	1	2	3	4	5	6	7.0	8.0	9.0	9.5	E10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16
Rat	Days	1	3.5	4-5	5	6	7.5	8.5	9	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5









Species	Stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Human	Days	1	2-3	4-5	5-6	7-12	13-15	15-17	17-19	20	22	24	28	30	33	36	40	42	44	48	52	54	55	58
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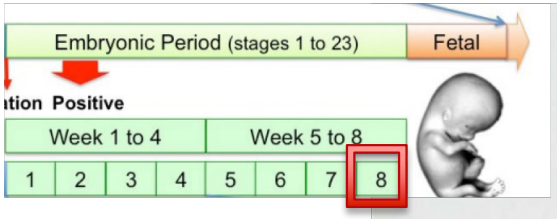
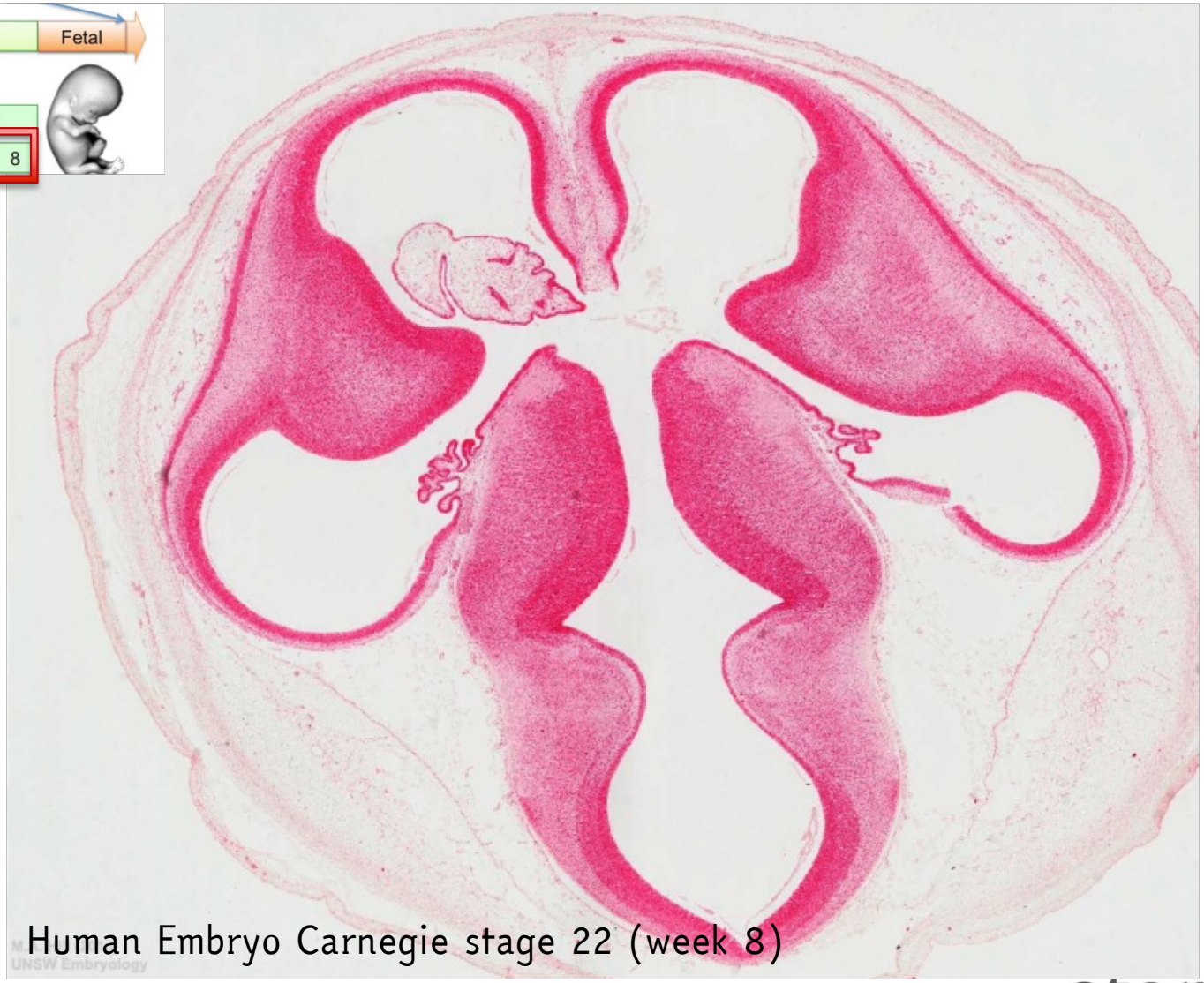
Rat	Days	1	3.5	4-5	5	6	7.5	8.5	9	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5





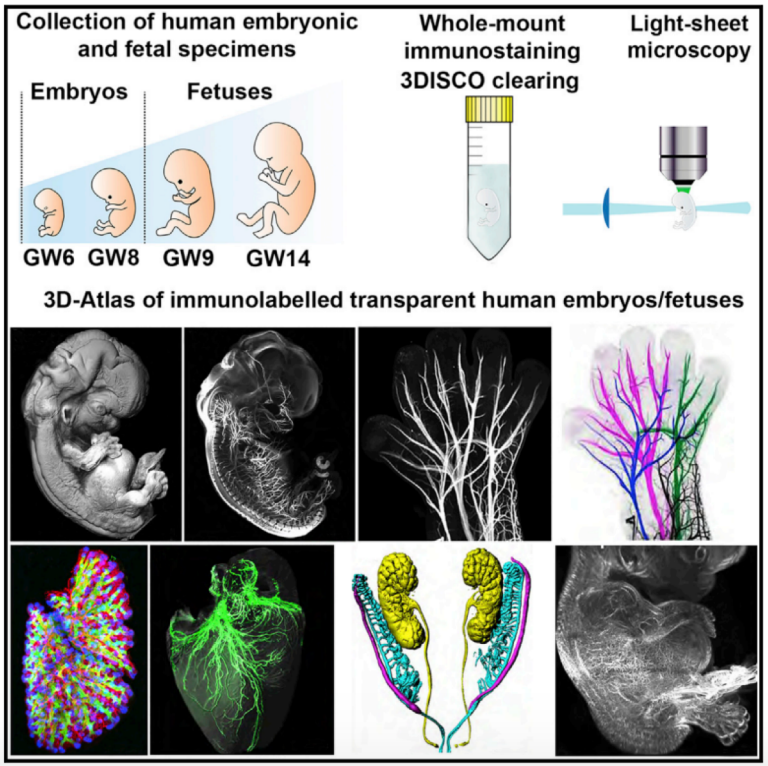
Human Embryo Carnegie stage 22 (week 8)

Resource

Cell 2017

Tridimensional Visualization and Analysis of Early Human Development

Graphical Abstract



Authors

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Correspondence

alain.chedotal@inserm.fr

In Brief

An initial look at dynamic processes during early human development through 3D cellular imaging.

https://transparent-human-embryo.com

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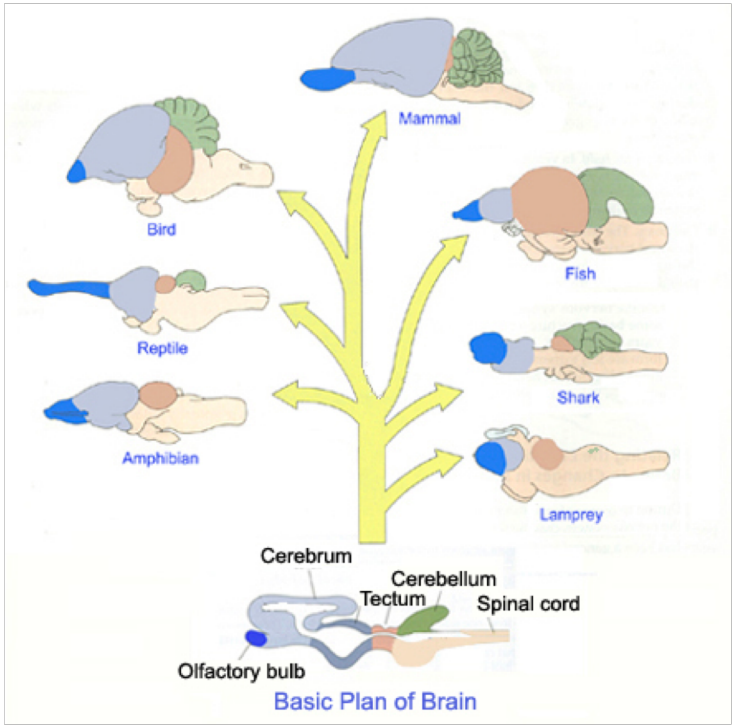
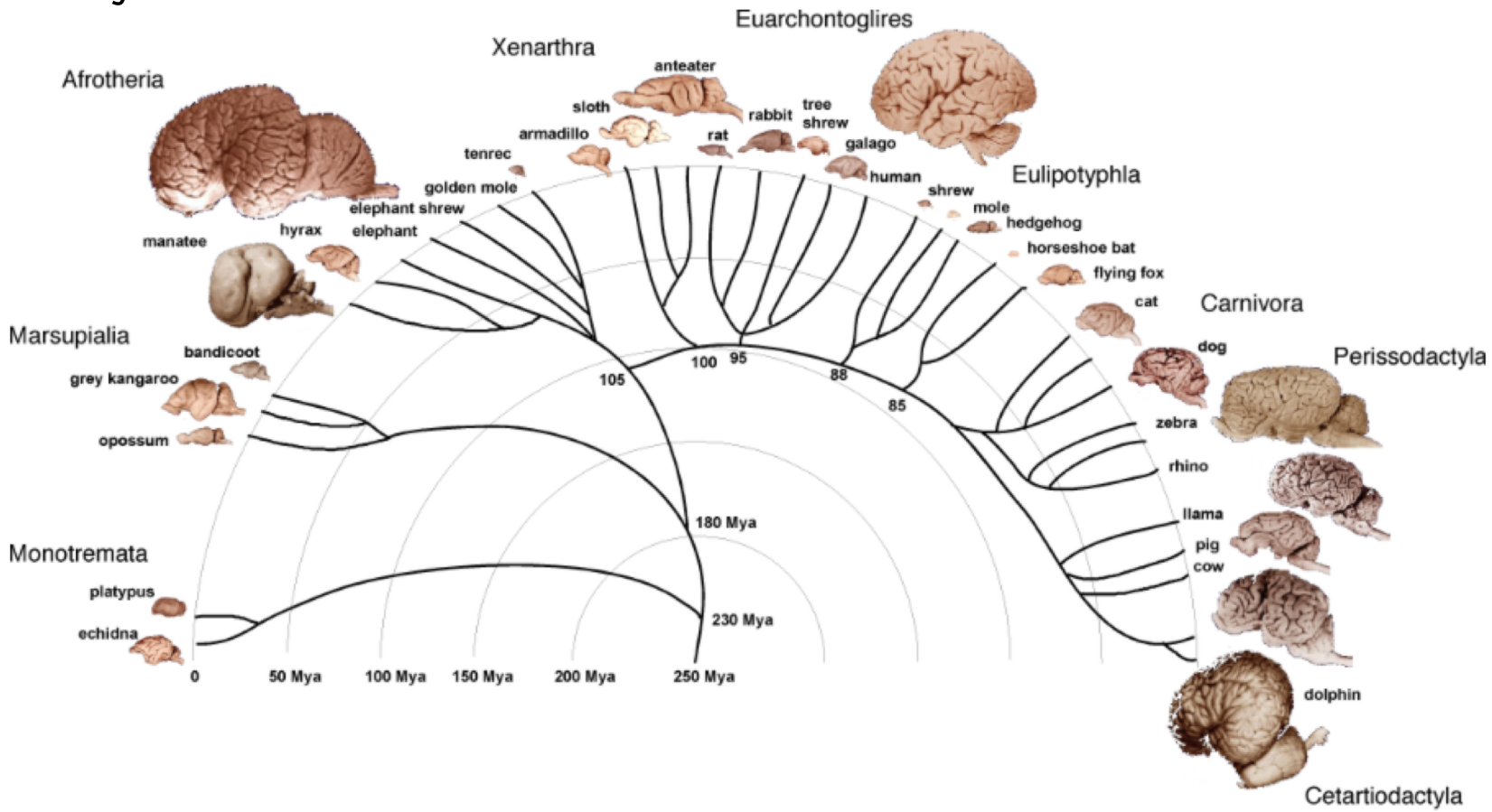
Great diversity in vertebrate adult brain forms



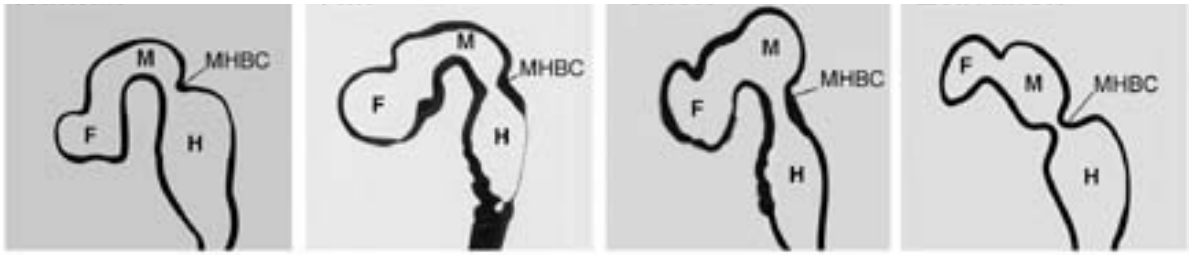
Brain diversity in mammals



From Herculano-Houzel (2012) PNAS

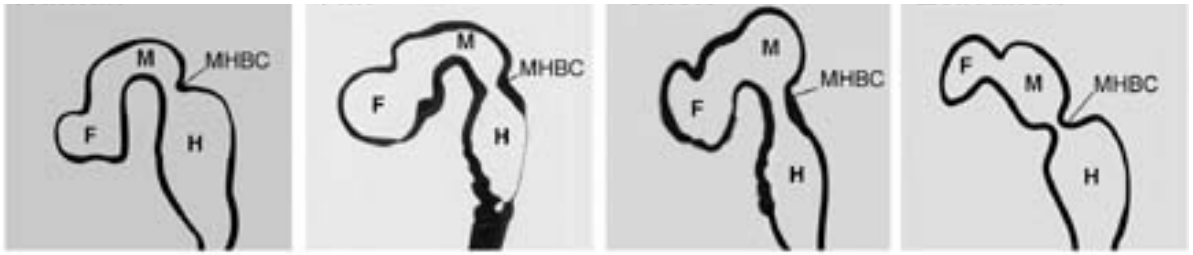
All vertebrate embryonic brain shortly after neurulation exhibit a very similar pattern of organization

? ? ? ?



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

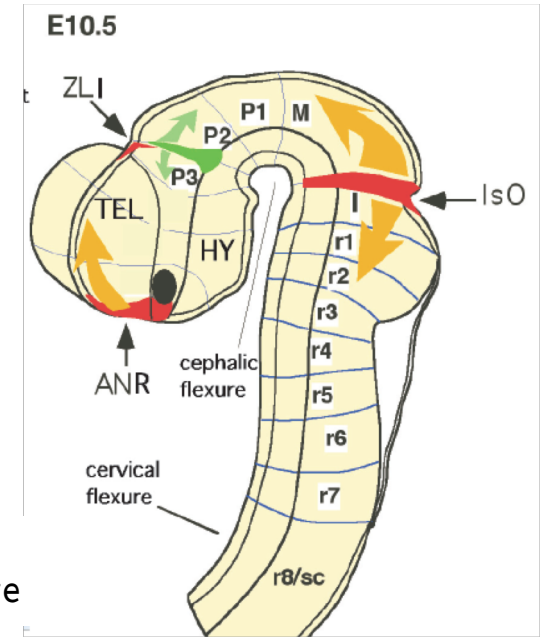
human rat chick zebrafish



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

Embryonic brain archetype (Bauplan)

Prosomeres
Mesomeres
Rhombomeres



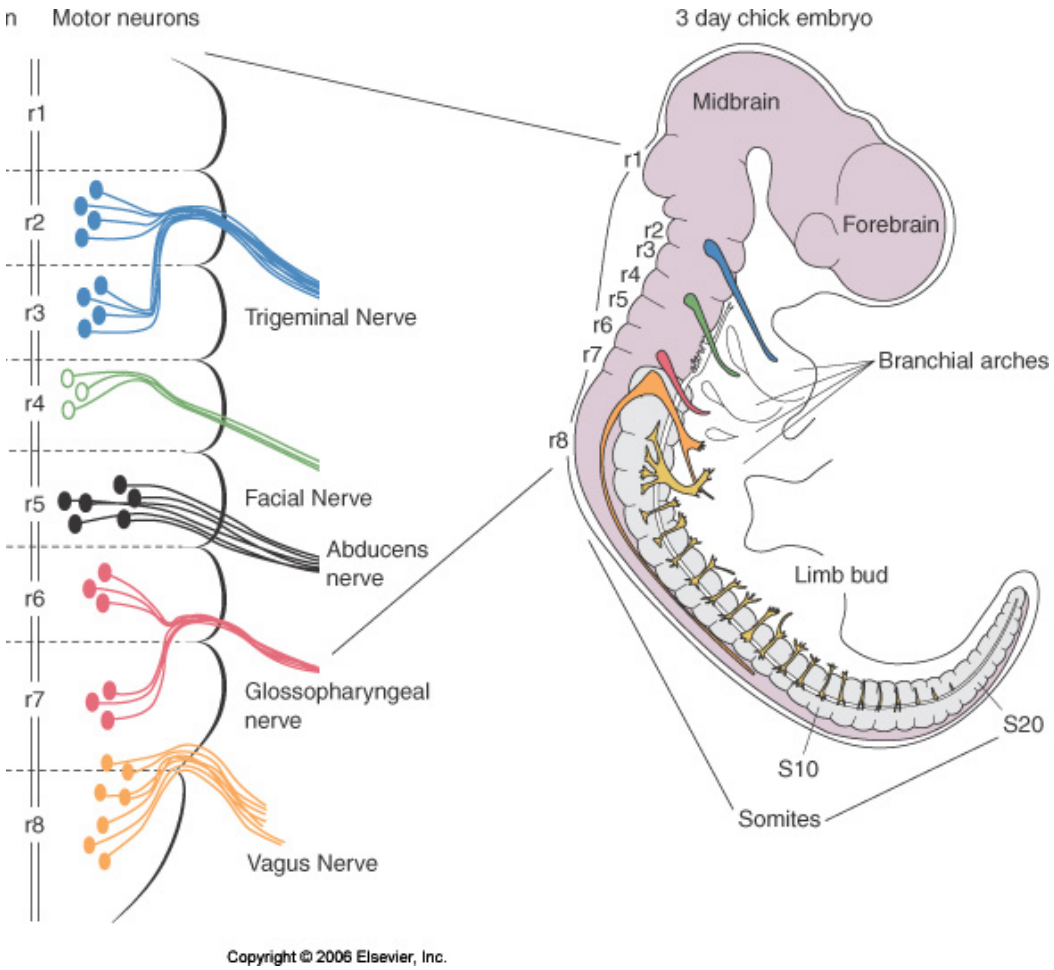
Vertebrate pharyngula stage

Neuromeres = series of embryonic segmental units, or cellular compartments, from which progenitors proliferate, migrate and differentiate forming the different parts of the vertebrate brain

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

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

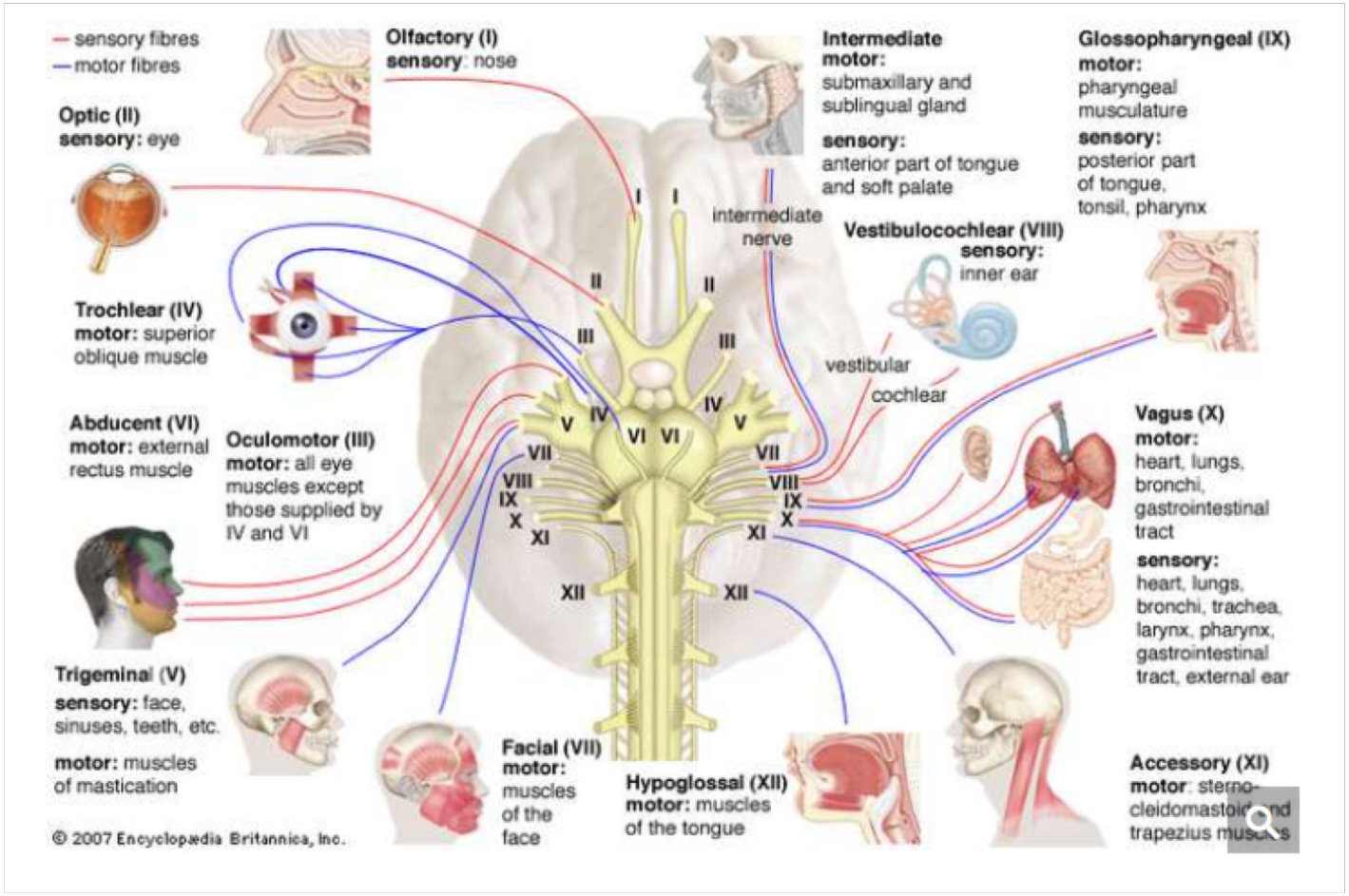
Rhombomers in the developing hindbrain



Hindbrain development involves the generation of eight neuroepithelial compartments or rhombomeres, each with a distinct identity according to its anteroposterior (A-P) position

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

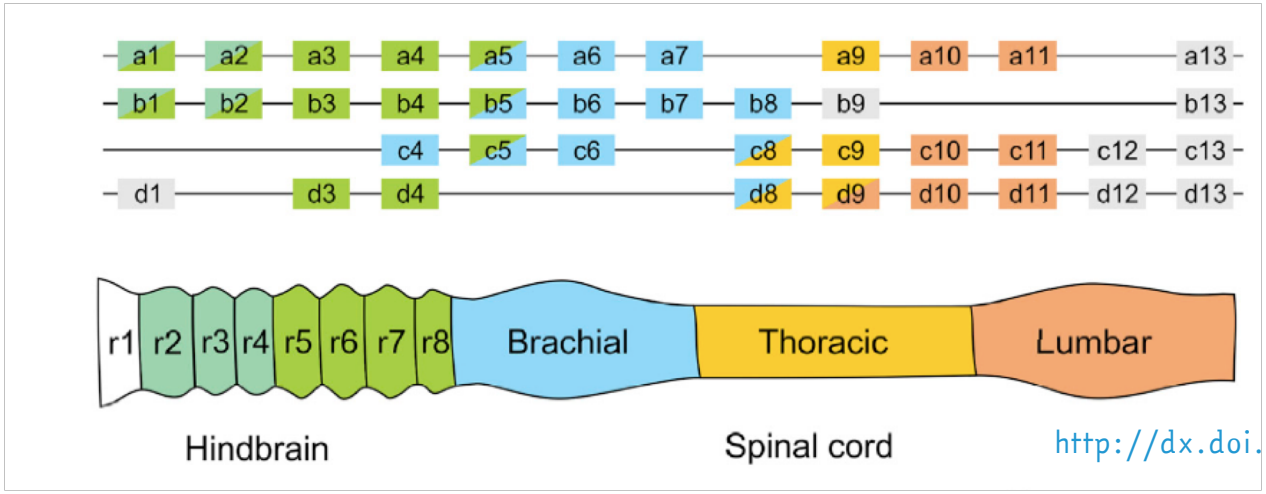


Hindbrain: region of the brain that coordinates motor activity, breathing rhythms, and many unconscious functions

How do rhombomers become different to each other?

Hox genes control the regional identity of the hindbrain and Spinal Cord

Color coding of Hox genes represents expression domains along the rostrocaudal axis.



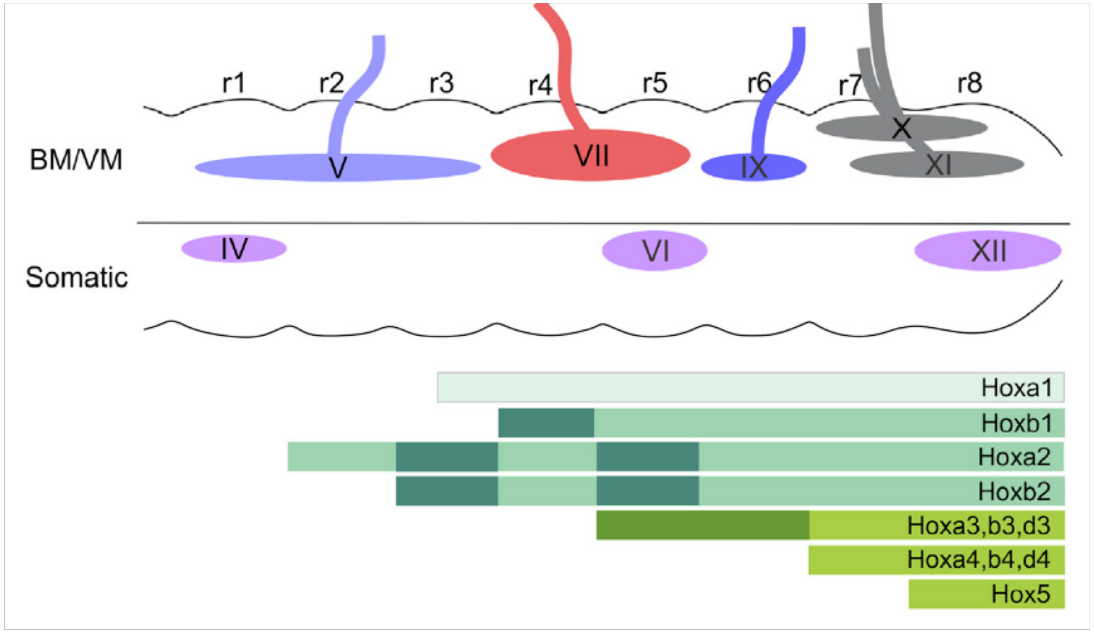
http://dx.doi.org/10.1016/j.neuron.2013.09.020

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

In most vertebrates, 39 Hox genes are distributed across four clusters (HoxA,B,C,D)
Each gene belonging to one of the 13 paralog groups (1-13)

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



http://dx.doi.org/10.1016/j.neuron.2013.09.020

In the hindbrain are expressed Hox genes from paralog groups 1–5

Anterior expression limits correspond to rhombomere boundaries

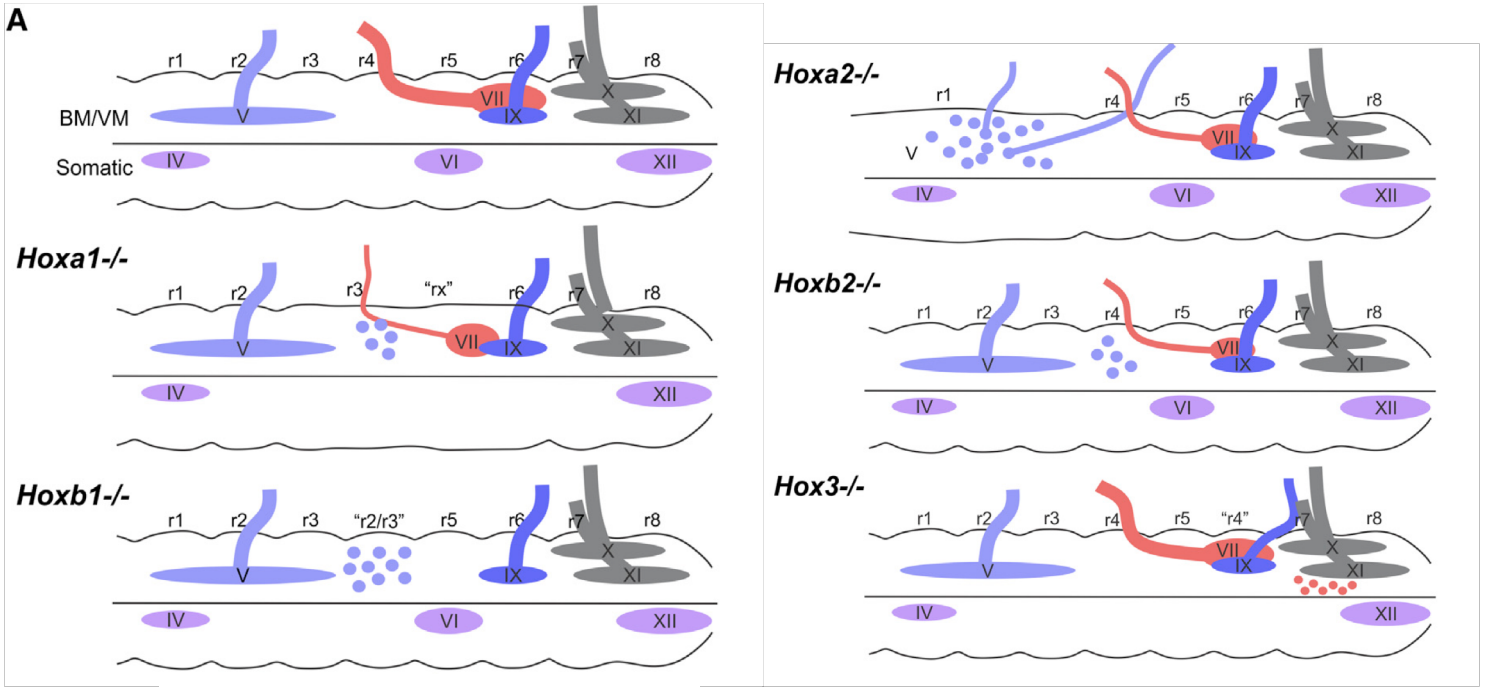
Higher color intensity denotes higher expression

Hoxa1 expression is transient

Hindbrain motor nuclei develop within specific rhombomeres and are shown within their rhombomeres of origin

IV, trochlear; V, trigeminal; VI, abducens; VII, facial; IX, glossopharyngeal; X, vagus; XI, accessory; XII, hypoglossal

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



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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

Ellen M. Carpenter, Judy M. Goddard, Osamu Chisaka, Nancy R. Manley and Mario 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

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

*These authors contributed equally to this work

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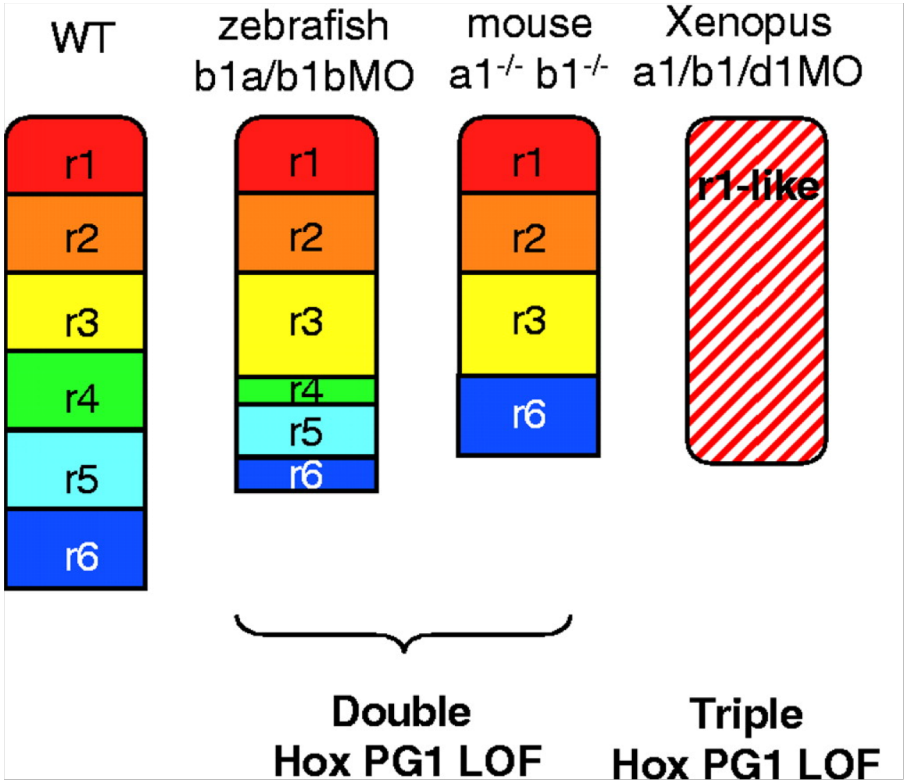
Development

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)


WT
zebrafish
b1a/b1bMO
mouse
a1-/- b1-/-
Xenopus
a1/b1/d1MO
r1
r2
r3
r4
r5
r6
r1
r2
r3
r4
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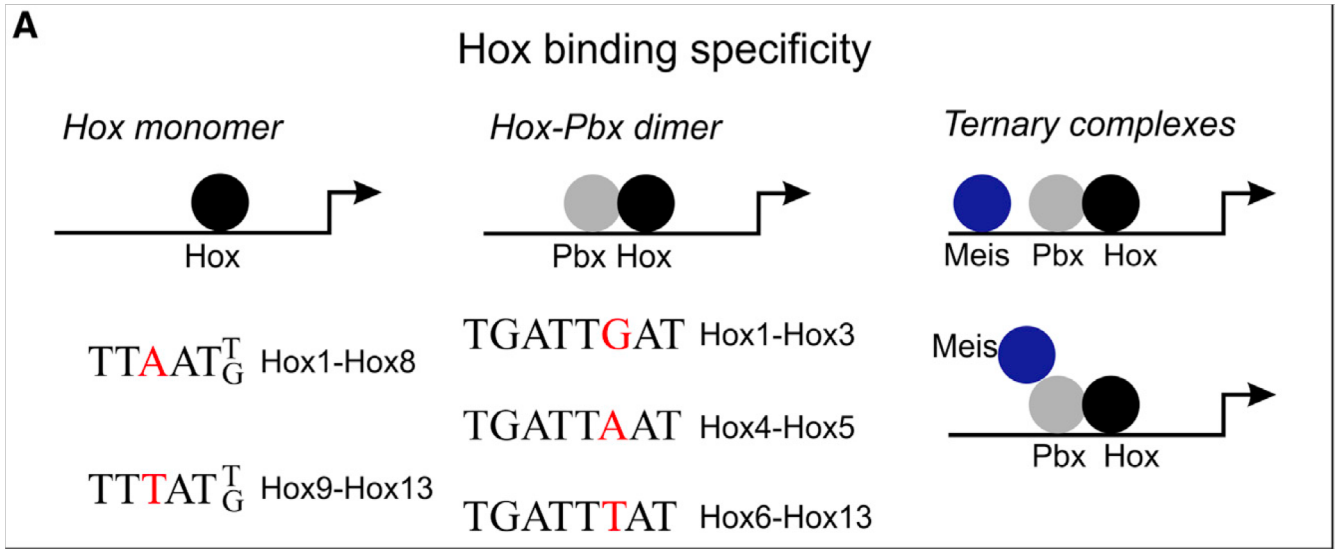
r6
r1
r2
r3
r6
r1-like
Double
Hox PG1 LOF
Triple
Hox PG1 LOF



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.

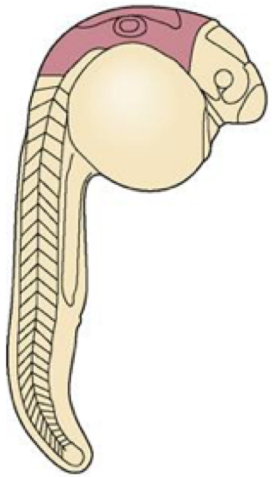
Waskiewicz AJ[1], Rikhof HA, Moens CB.

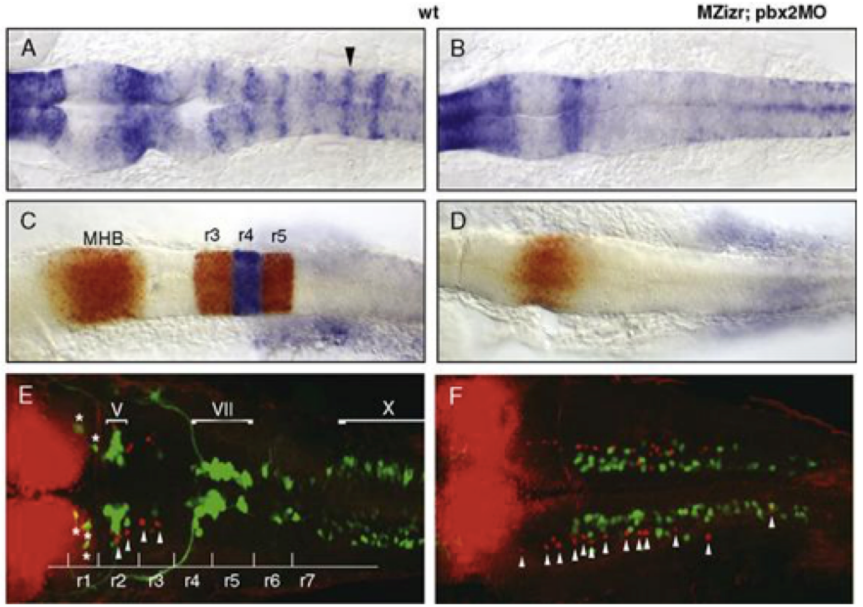
⊕ **Author information**

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

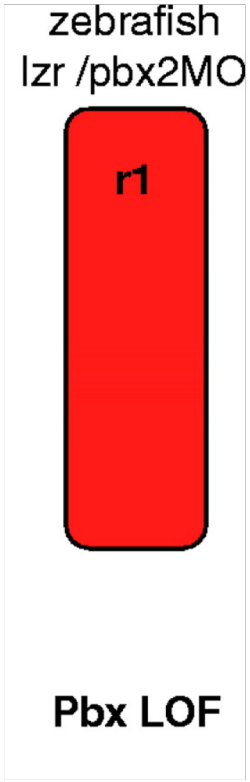




Gene expression

Neuronal types

r1 is a hindbrain ground ("default") state

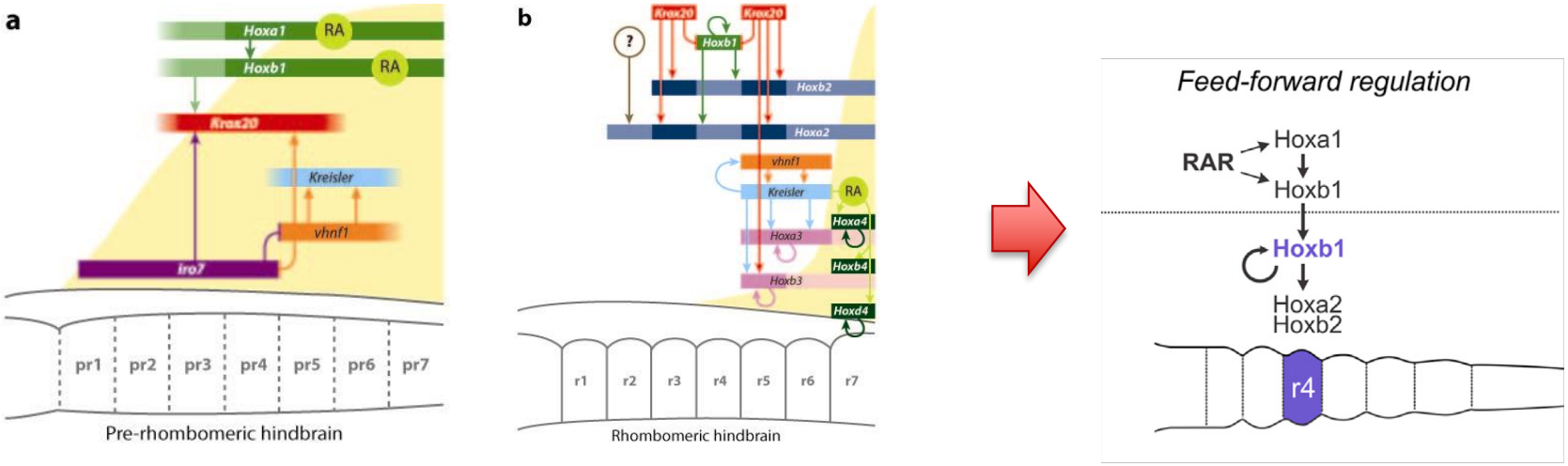


What control the patterned expression of Hox genes in the hindbrain?

What control the patterned expression of Hox genes in the hindbrain?

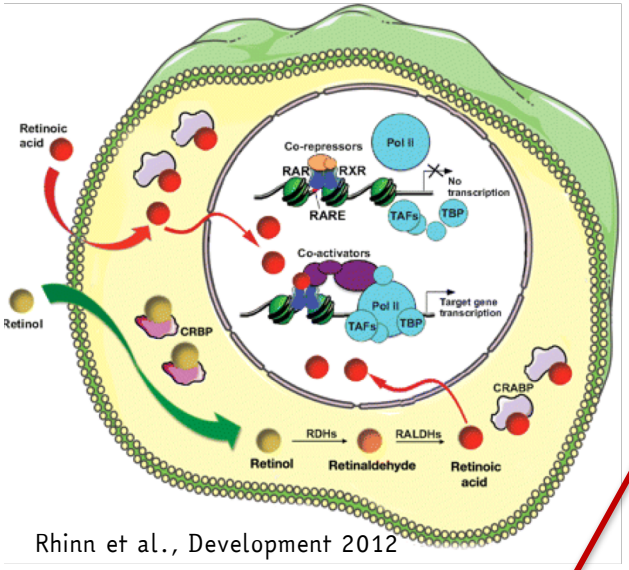
- The initial inductive phase involves the sequential activation of genes within a Hox cluster and is mediated by **Retinoic Acid** acting in a graded manner along the rostrocaudal axis

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



Krox20 represses Hoxb1 in the adjacent rhombomeres

Alexander et al., 2009 Annu Rev Cell dev Biol

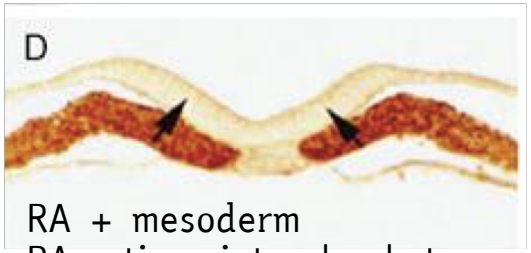


Rhinn et al., Development 2012

RA

Stem cell differentiation (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)

Segmentation and patterning of the hindbrain
(RA controls gene expression in a concentration dependent manner)



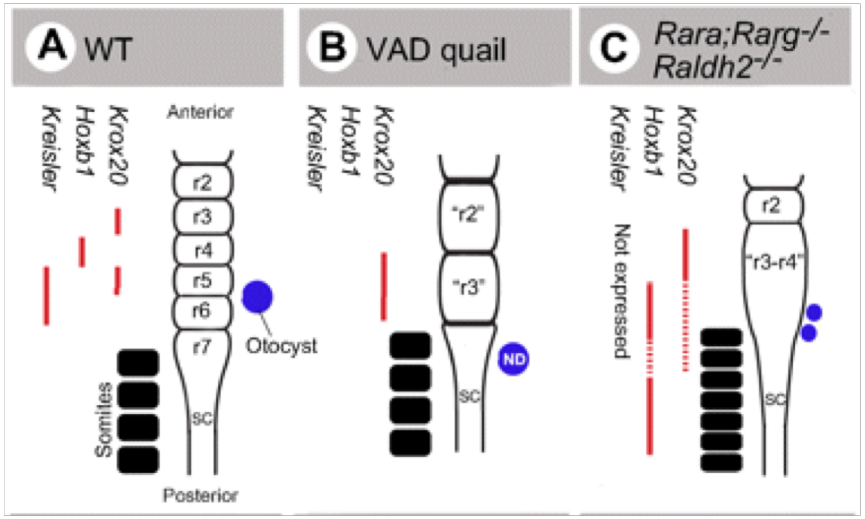
RA + mesoderm
RA action: interplay between diffusion (morphogen-like) gradients and the establishment of signalling boundaries due to RA metabolism

Teratogenic

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

(Craniofacial and brain abnormalities)

Hindbrain abnormalities in animal models with altered RA signalling

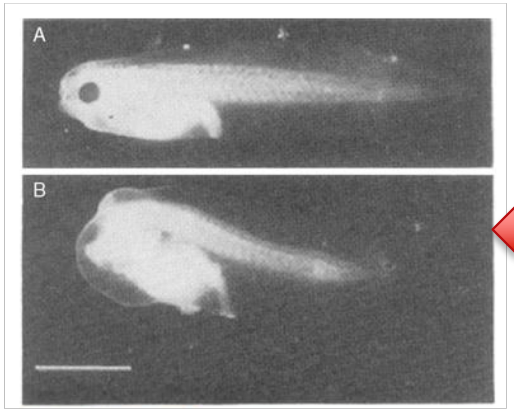


animal models **with endogenous deficiency in RA signalling**:

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

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

Raldh2 = enzyme that catalyzes RA syntesis From retinaldehyde



RA-treated Xenopus embryo

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...)