Regionalization of the nervous system

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7.68J/9.013J

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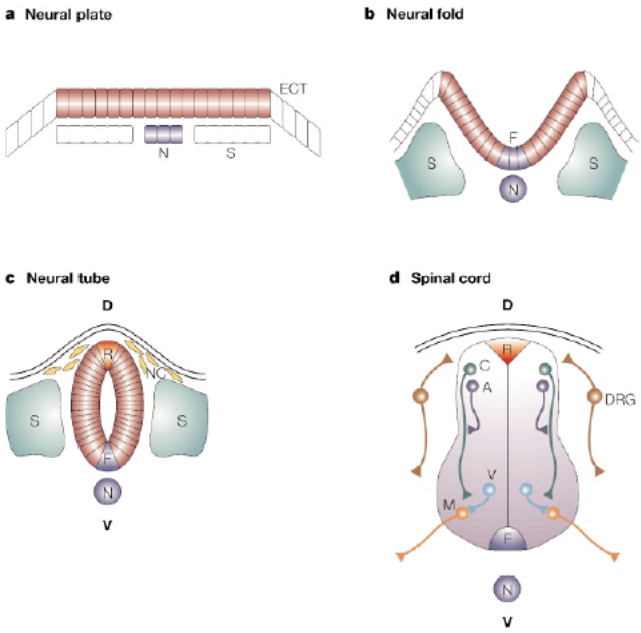
Patterning along:

- Rostral/Caudal (AP) axis
- Dorsal/Ventral (DV) axis
  - Start with DV axial patterning in Spinal Cord

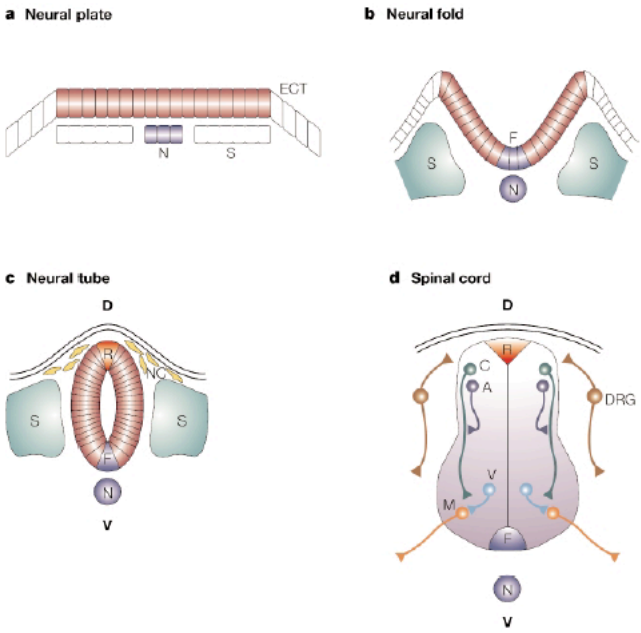
Dorsal/Ventral Axis patterning

- **Structures along DV axis of Neural Tube**
  - **Roof plate (R)**
  - **Floor plate (F)**
  - **Notochord (N)**
  - **Neural crest (NC)**
  - **Paraxial mesoderm/somites (S)**



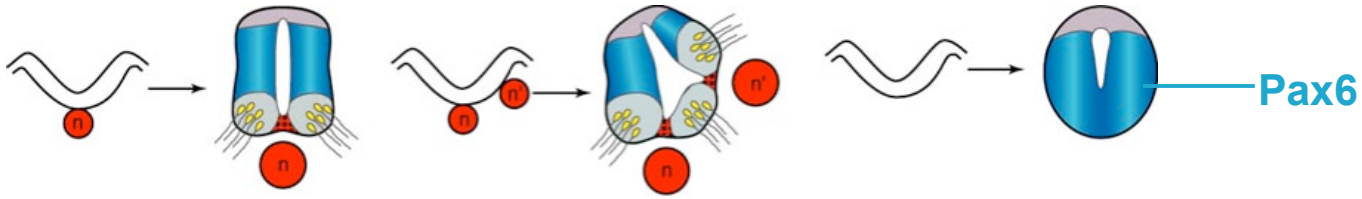
Dorsal/Ventral Axis patterning

- **Structures along DV axis of embryonic spinal cord**
  - **Dorsal:**
    - **Commissural Neurons (C)**
    - **Association Neurons (A)**
  - **Ventral**
    - **Ventral Interneurons (V)**
    - **Motor Neurons (M)**
  - **Dorsal Root Ganglion (DRG) Neurons -- Neural crest - derived**



The Notochord in patterning DV axis

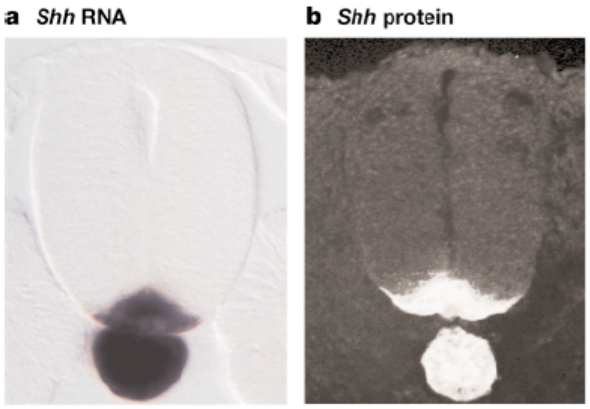
- **Notochord :**
  - **remove notochord --- lose ventral cells types (floor plate and motor neurons), ventral cells assume more dorsal fates [pax6])**
  - **Transplant in second notochord --- generate ectopic floor plate and motor neurons**



- **Notochord: produces inductive signal(s) --- can induce ventral fates --- floor plate later acquires similar inductive ability**
- **Neural tube cells: competent to respond to inductive signals by assuming different DV fates**

What is the molecular nature of the inductive signal?

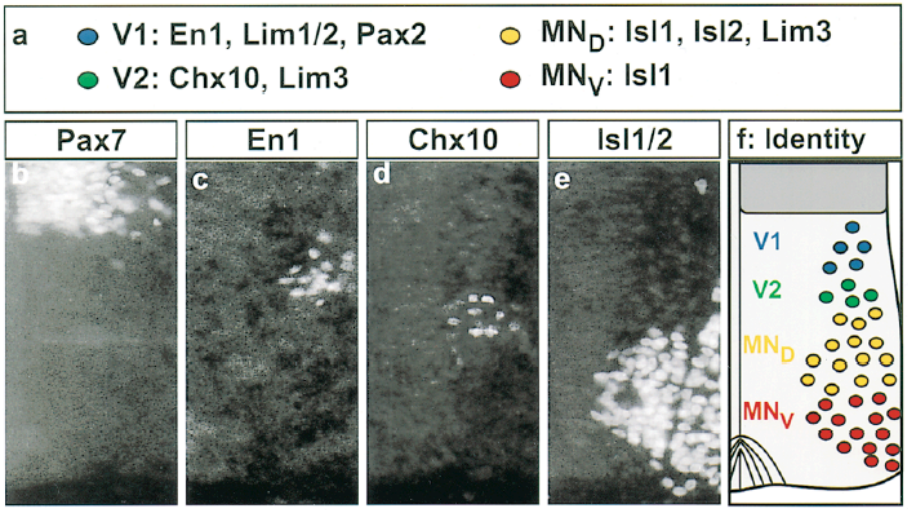
- **Secreted protein : Sonic hedgehog (Shh)**
  - **Produced by notochord and floor plate**
  - **Ectopic Shh induces ectopic ventral cell types (floor plate, motor neurons, ventral interneurons)**
  - **Can have effects on cells several diameters from source**
  - **Sonic hedgehog knockout mice lack most ventral cell types (floor plate, motor neurons, most ventral interneurons)**

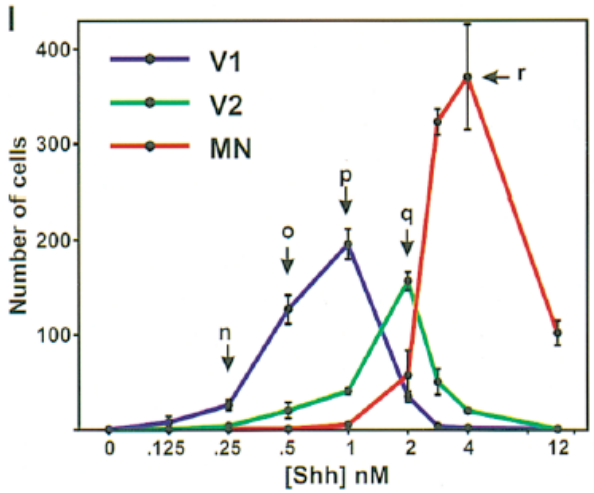


chick spinal cord

Shh can promote differentiation of distinct cell types at different concentrations

- Treat explants of chick neural plate with different concentrations of Shh
- 10 nM Shh --- neural plate cells become floor plate
- 1-4 nM Shh -- become motor neurons
- 0.1- 1nM Shh -- become ventral interneurons
  - Compare p, q and r --- 2-fold changes, large change in outcome

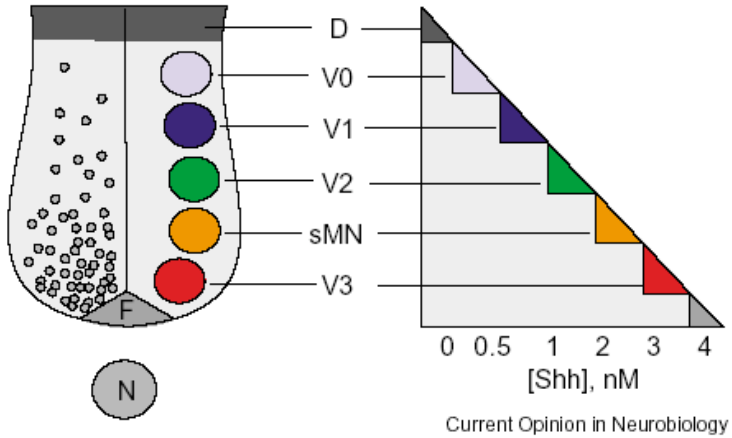




Ericson et al Cell 90:169 (1997)

Shh can act as morphogen

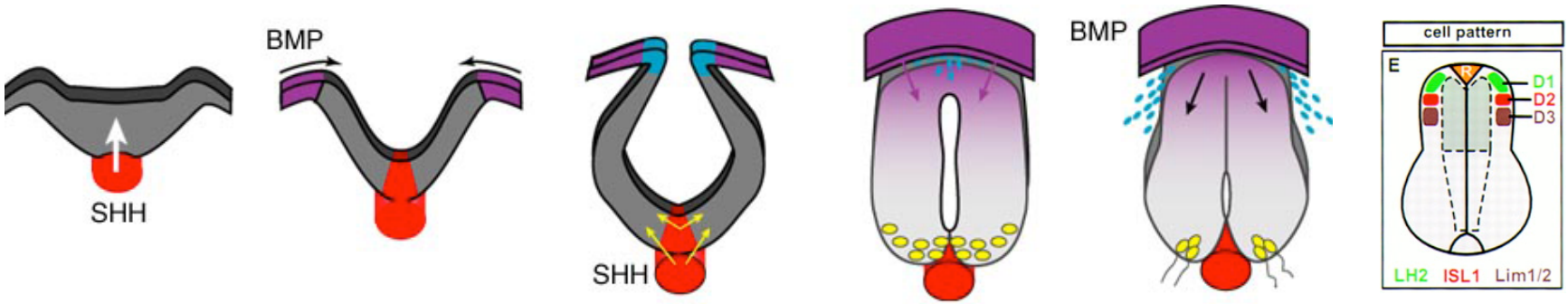
- There are multiple concentration thresholds for Shh signaling --- not just plus/minus
- Special type of inducer: generates graded response ---morphogen
- Shh: Higher concentration Shh more ventral fate
- Shh may act in graded fashion over long range to pattern V cells



Briscoe and Ericson, Curr. Opin. Neur. (2001) 11:43.

Patterning of Dorsal Spinal Cord

- Dorsal neural development is independent of ventral induction
  - Eg., remove notochord -- still get dorsal markers
- Dorsal inductive signals:
  - Early: epidermal ectoderm flanking lateral edges of neural plate (planar)
  - Later: surface ectoderm contacts dorsal neural tube
- BMP's are important for this induction
  - Expressed in epidermal ectoderm
  - Can mimic ability of epidermal ectoderm to induce roof plate, neural crest, and dorsal interneurons

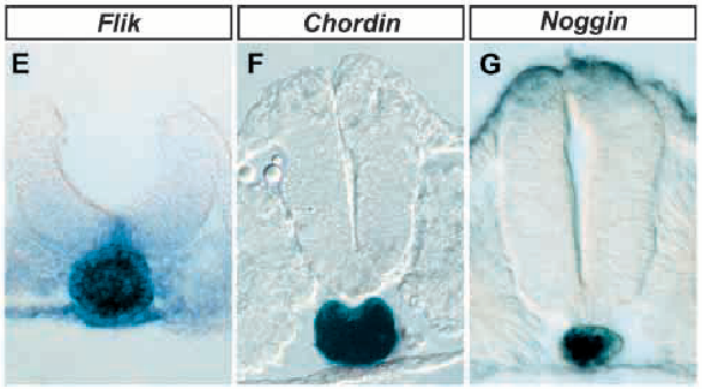


Specification of different dorsal fates

- **Multiple BMP's expressed in nested pattern -- could act in combinatorial fashion on cells**
- **BMP's could act in graded fashion:**
  - **Zebrafish bmp2 mutant -- lose dorsal fates as ventral fates expand**
  - **Some concentration-dependent differences *in vitro***
- **Competence of cells to respond to signals may also be important:**
  - **dorsal-most fates [roof plate and neural crest] determined early (neural tube closure), interneurons later --**
  - **Can recapitulate this in vitro --- newly formed neural plate cells + BMP's yield neural crest; if mature neural plate in vitro for 24h then add BMP's get interneurons**

Interaction between Dorsal and Ventral induction programs

- **BMPs shift *in vitro* response to constant concentration of Shh:**
  - **fates become more dorsal**
- **BMPs can antagonize responses to Shh -- mechanism?**
- **Ventral signals inhibit BMPs: Notochord produces BMP antagonists**

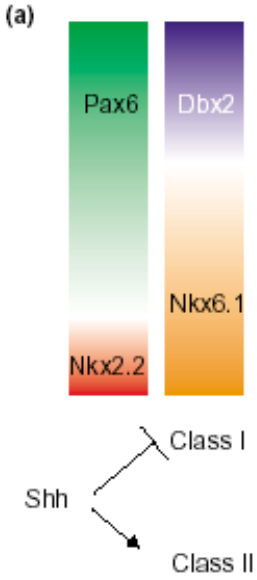


Interpreting gradations of Shh

- Progressive 2-3 fold changes generate 5 distinct ventral neuron types
- Neurons at more ventral locations require more Shh, neurons at less ventral locations require less
- DNA-binding transcriptional regulatory proteins are key intermediaries in mediating the dosage-sensitive response

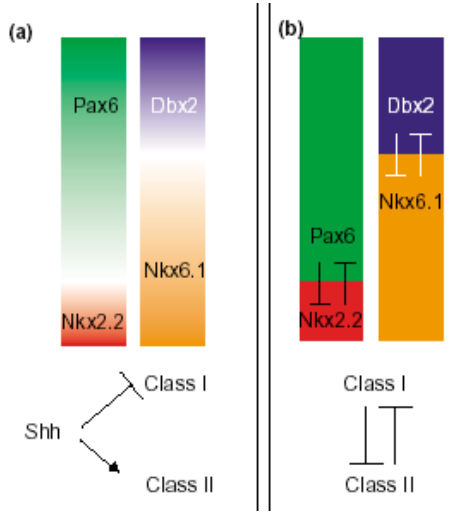
Current model for dosage-sensitive response to Shh

- **Mediated through two classes of transcription factors:**
  - **Class 1: repressed by Shh - in concentration-dependent fashion (Pax6, Dbx2)**
  - **Class 2: activated by Shh - in concentration-dependent fashion (Nkx6.1, Nkx2.2)**



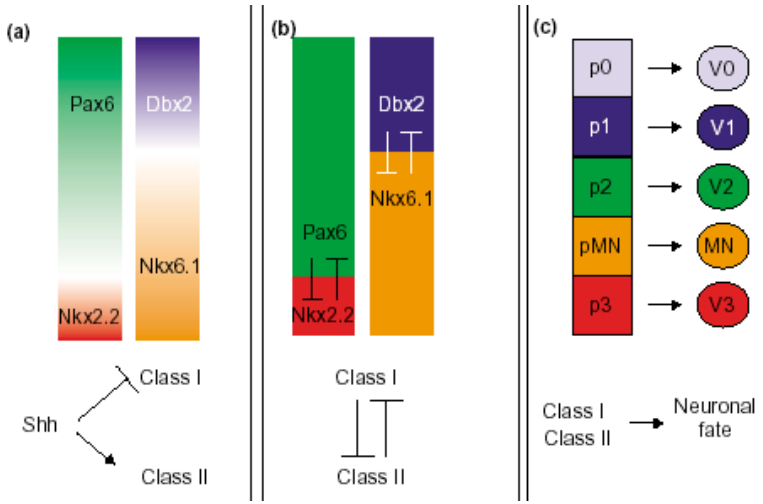
Cross-repressive interactions may sharpen boundaries

- Class I and Class II transcription factors regulate one another's expression
- Sharp boundaries can generate discrete responses



Three-step model for Ventral fate specification by Shh

- Class I and Class II transcription factors regulate one another's expression
- Sharp boundaries can generate discrete responses
- Different combinations of factors specify distinct identities



Open questions

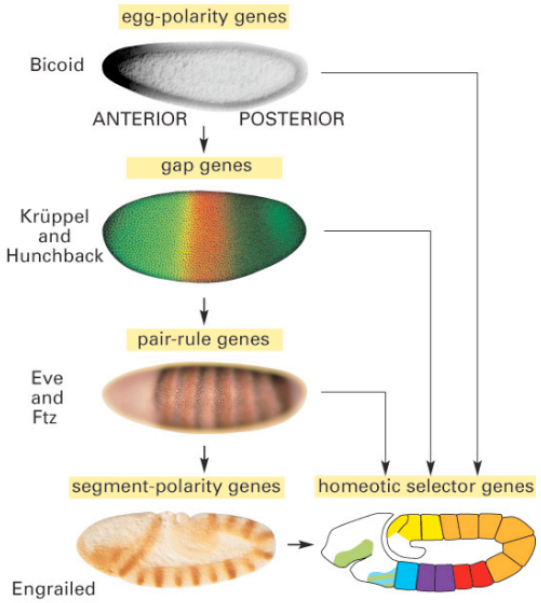
- Does Shh directly control Class I and/or Class II genes?
- Do Class I and Class II genes directly regulate one another?
- How is hedgehog signal transduced?

Precedent for graded responses in *Drosophila*

- ***Drosophila*** **AP axis specified by gradient of Bicoid**
- **Bicoid protein high at anterior, low at posterior**
- ***Bicoid*** **loss of function --- lose anterior structures**
- **Extra doses of Bicoid gene --- anterior structures expand toward posterior**

Early AP patterning in *Drosophila* : Progressive subdivision of embryo

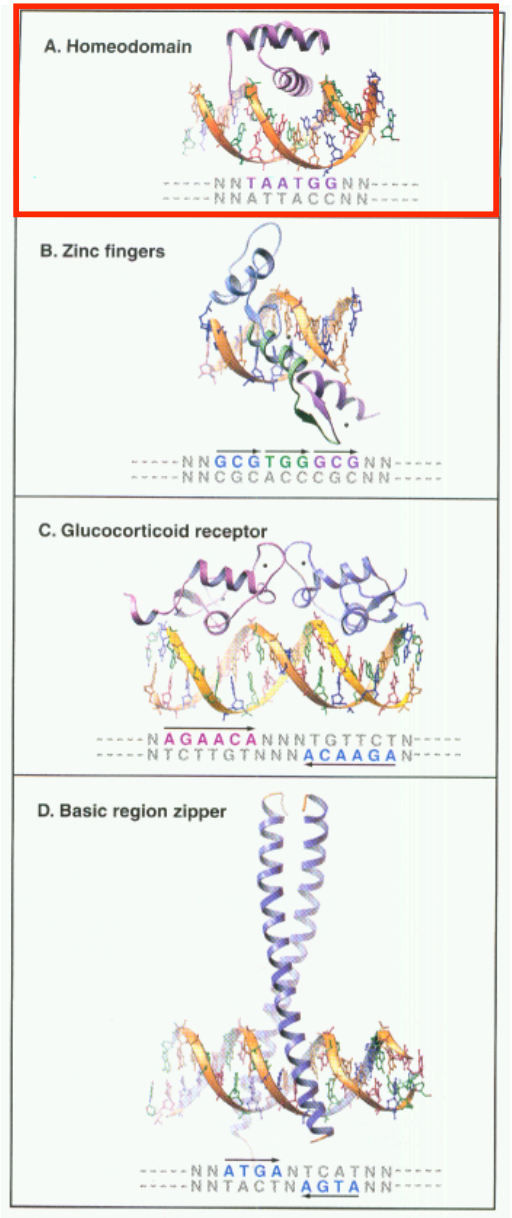
- Different levels of Bicoid activate different “Gap genes” in different regions along AP axis
- These gap genes cross-regulate one another to set up sharper boundaries
- Gap genes act in combination to regulate downstream pair-rule genes --- which are expressed in narrower regions
- Segment-polarity genes are targets of pair-rule genes --- yielding even finer regional regulation
- Sets up pattern of homeodomain-containing homeotic selector genes



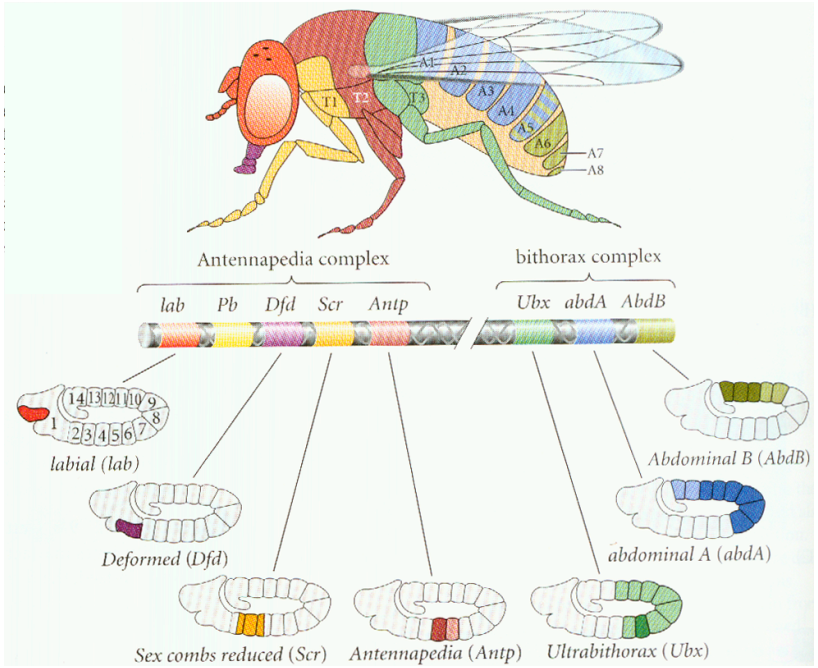
Homeodomain proteins

- DNA-binding transcriptional regulators
- Contain 60 amino acid domain that binds DNA in sequence-specific fashion
- Different homeodomain proteins have some overlap in DNA-binding specificity ----
  - Associate with other transcriptional regulatory proteins --- confers additional specificity for targets --- such combinatorial specificity common theme in development

Common transcription factor families

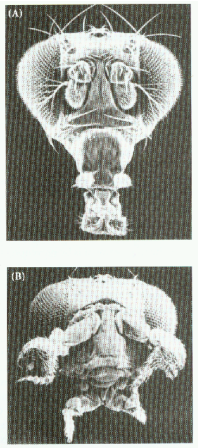


Homeotic selector genes are expressed in specific patterns along AP axis



Homeotic selector genes specify AP identity

- **Ectopic expression of homeotic gene can cause transformation of AP identity**
  - **Homeotic selector gene Antennapedia (Antp) normally expressed in abdominal region that gives rise to legs**
    - **If misexpressed in presumptive antennal region get legs on head**



wild type

Antp gain-of-function

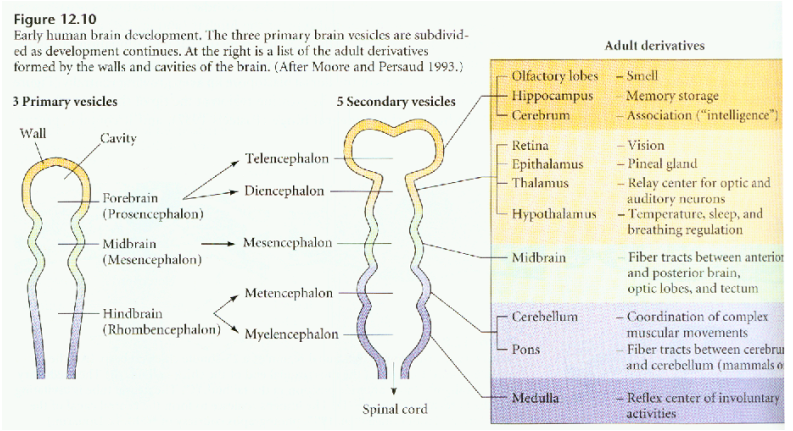
Homeotic selector genes specify AP identity

- **Loss of homeotic genes: embryo lose distinctions between different AP positions**
  - **Homeotic selector gene Ultrabithorax (Ubx) normally expressed in abdominal region that gives rise to halteres (balancers) behind wings -**
    - **loss of expression --- halteres transformed into wings --- four-winged fly**

wild type **Ubx loss-of-function**

Neural patterning along AP axis

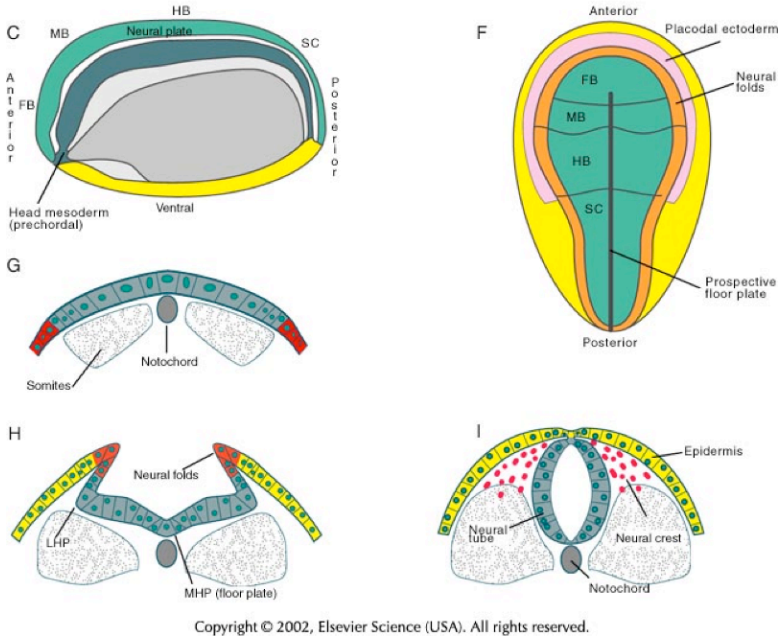
- **Vertebrate embryo:**
  - **Differential rates of proliferation lead to three brain vesicles**
  - **Prosencephalon (forebrain)**
    - **Telencephalon**
    - **Diencephalon**
      - **Thalamus, hypothalamus, optic vesicles**
  - **Mesencephalon (midbrain)**
  - **Rhombencephalon (hindbrain)**
    - **Metencephalon -- cerebellum**
    - **Myencephalon -- medulla**
  - **Spinal cord**



S.F. Gilbert, Developmental Biology, 6th edition, (2000) Sinauer.

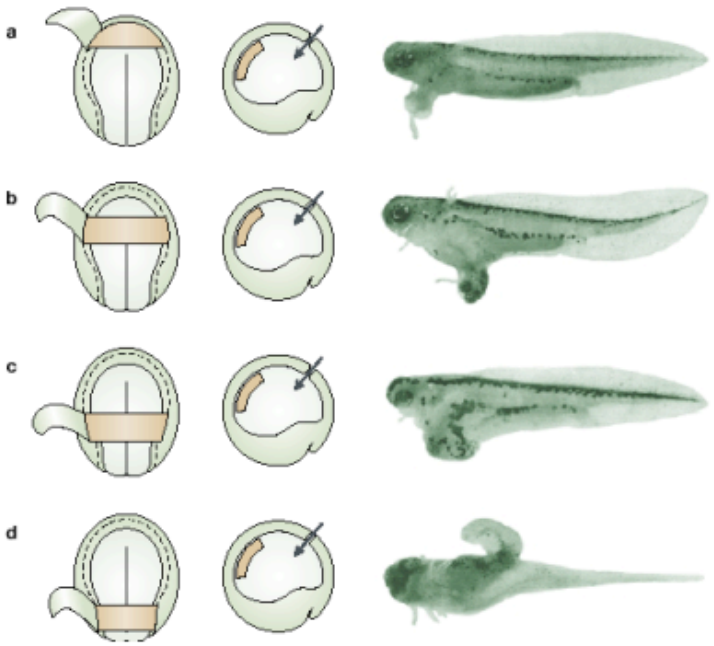
Early AP patterning in vertebrates:Neurulation

- **Bending of neural plate**
  - **Accompanied by convergence-extension along AP axis**
- **Dorsal edges fuse to form neural tube**
- **A/P axis differences already apparent**



Organizer-derived cells pattern the nervous system along the AP axis

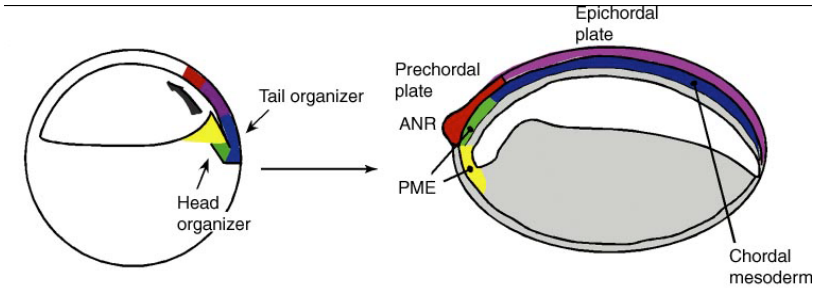
- Descendents of organizer cells come to lie beneath the neural tube along its length (archenteron roof)
- Otto Mangold (1933) transplanted four successive regions of the archenteron into blastula
- Anterior pieces induced anterior structures (balancers, eyes)
- Posterior pieces induced posterior structures (hindbrain,tail mesoderm)



Stern (2001) Nat. Rev. Neurosci. 2: 92

Two-step model for AP axis production

- Organizer has distinct along AP axis:
  - Head :will become Prechordal mesoderm (PME)
  - Tail: will become Chordal mesoderm (notochord and somites)
- "Activation" --- Initial neural induction associated with forebrain properties
- "Posteriorization" --- additional signals promote caudal character

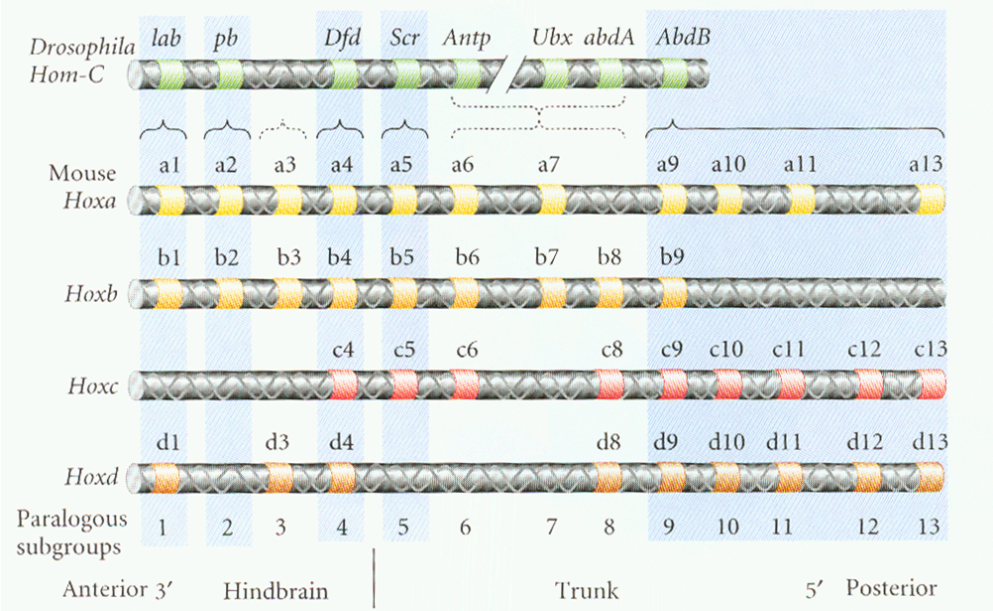


Two-step model for AP axis production

- **"Activation" --- Initial neural induction associated with forebrain properties --**
  - **antagonists of BMP signaling promote forebrain differentiation**
  - **Anterior mesoderm produces BMP inhibitors**
  - **Lose anterior region in mouse noggin and chordin mutants**
- **"Posteriorization" --- additional signals promote caudal character --**
  - **FGF (receptor tyrosine kinase ligand), WNT, and Retinoic Acid can all promote posterior from anterior structures**
  - **All are expressed in chordal mesoderm**

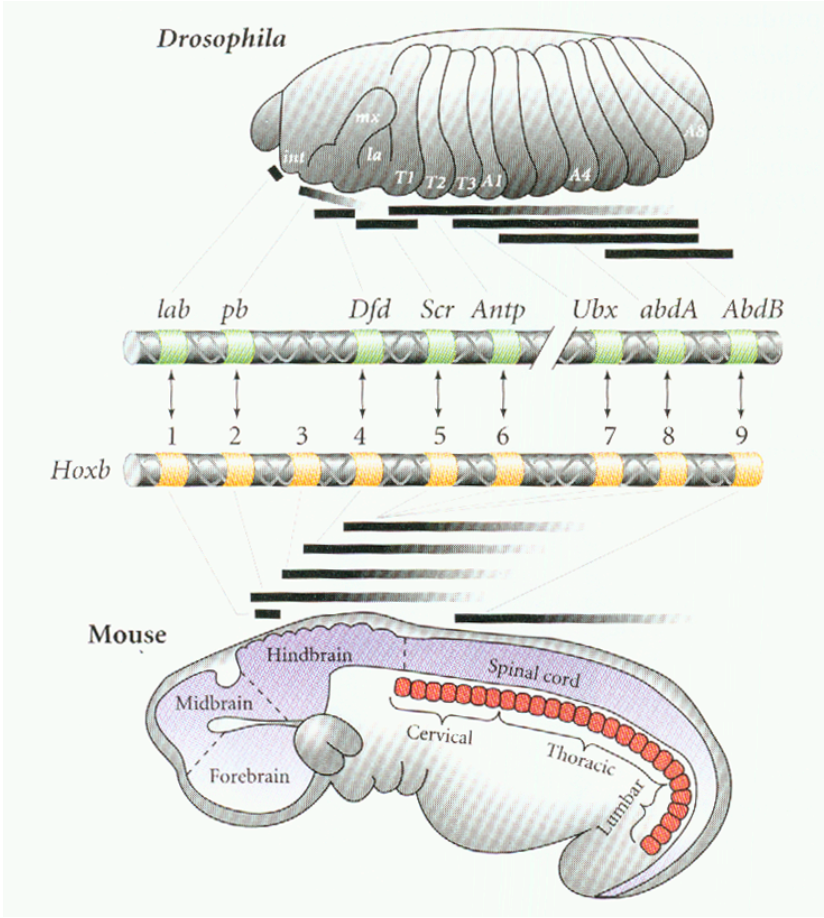
Homeodomain proteins also pattern AP axis in vertebrates

- **Mammals contain 4 clusters of homeodomain proteins (Hox proteins) related to the fly homeotic selector genes**



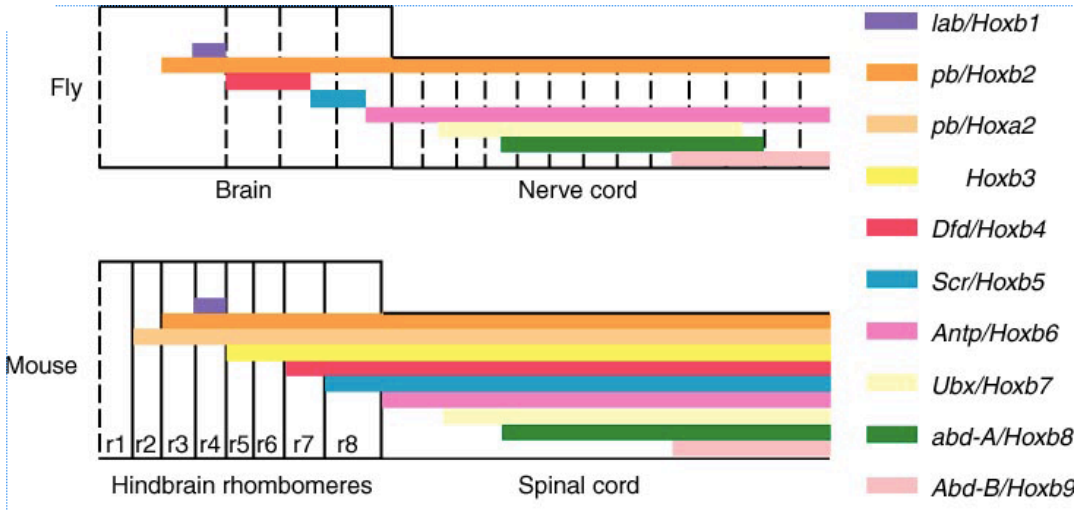
Hox genes show restricted expression patterns along AP axis from hindbrain to spinal cord

- Related genes in different species show related AP expression domain
- AP expression domain correlated with position in cluster

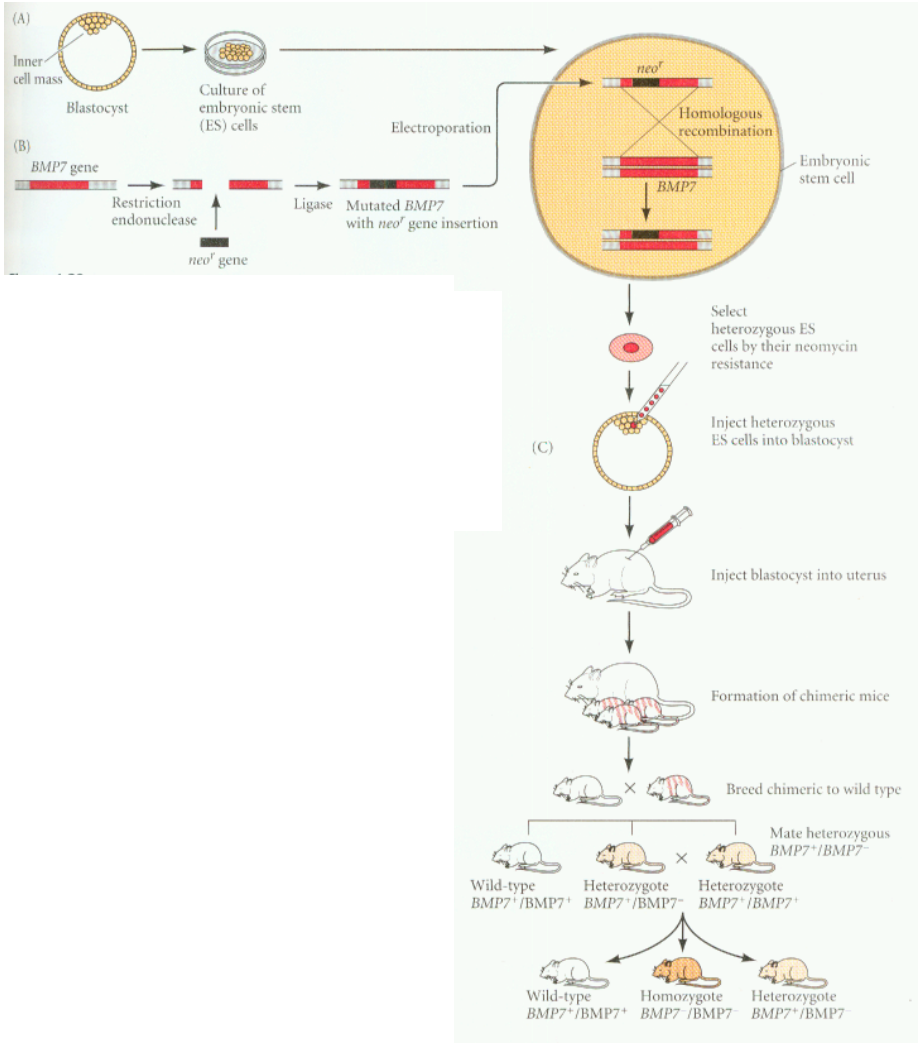


Hox genes and hindbrain patterning

- **Hindbrain: indentations demarcate modular organization along AP axis: each segment termed a rhombomere**
- **Different combination of Hox genes expressed in each rhombomere**
- **Each rhombomere has different fate**



Production of a targeted mouse knockout



S.F. Gilbert, Developmental Biology, Sixth Edition (2000) p.99

Hox genes contribute to hindbrain AP patterning

- Hoxb-1 normally highly expressed in rhombomere 4
- Hoxb-1 knockout mouse: rhombomere 4 (r4) now resembles rhombomere 2 (r2)
  - r4 motor neurons normally migrate caudally
  - In Hox-b1 knockout migrate laterally (like r2 motor neurons)

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Homeodomain proteins also pattern anterior nervous system

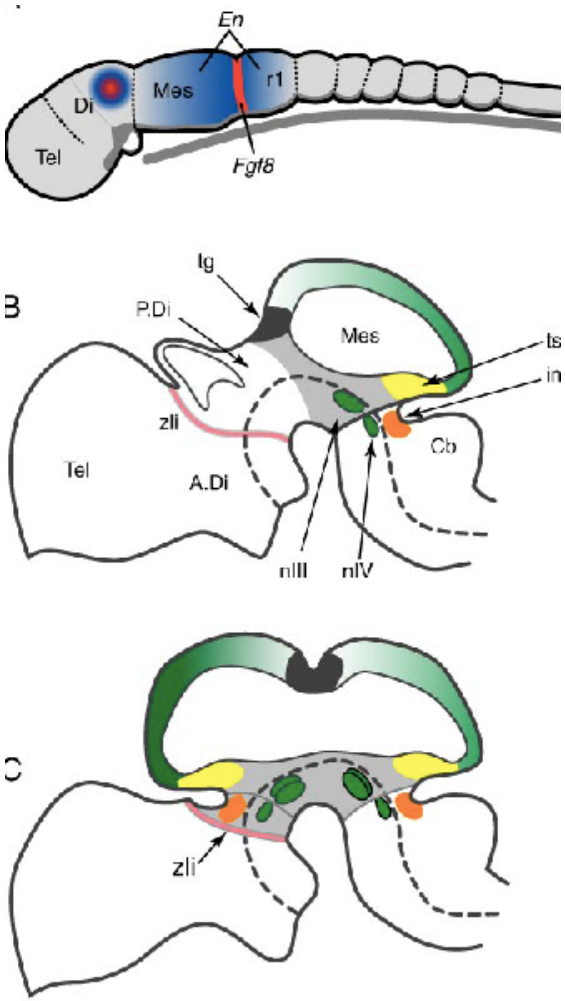
- Anterior to r2 express non-Hox homeodomain proteins
- Midbrain/hindbrain boundary: En1 and En2 (homologs of fly segment-polarity gene *engrailed*)
    - Lose this region in En1 knockout mice
- Forebrain: Emx1 and Emx2 (homologs of fly *empty spiracles* -- gap gene that patterns fly head/brain)
- Forebrain/midbrain: Otx1 and Otx2 (homologs of fly *orthodenticle* -- gap gene that patterns fly head/brain)
    - Otx2 knockout -- lose all head anterior to r3
    - Otx2 ectoderm-specific knockout -- forebrain converted to hindbrain --enlarged cerebellum at anterior of CNS

How is pattern of Hox gene expression set up in hindbrain?

- Retinoids can help specify Hox expression
- Retinoic acid: vitamin A derivative
- Acts via Retinoic acid receptors (RAR's):
  - RAR's: Ligand-dependent DNA-binding transcription factors -- bind promoters of target genes
- RA promotes posterior fates
  - Overexposure leads to expansion of posterior Hox genes into anterior regions and accompanying transformations of rhombomere fates --
  - Posterior high/ anterior low?
    - RA produced by somites near r7/r8 who express Raldh2 (RA synthetic enzyme)
      - Raldh2 knockout mice -lose posterior Hox gene expression, anterior Hox genes expressed only in posterior hindbrain
    - RA degraded by anterior regions that express Cyp26

Midbrain/anterior hindbrain patterning

- **En1 and En2 expressed across boundary in graded fashion -- highest at boundary**
- **Fgf8 expressed on posterior side (r1 hindbrain --- cerebellum)**
  - **Implant Fgf8 bead into presumptive diencephalon -- transform into midbrain**
  - **AP polarity reversed -- mirror-image En gradient?**
  - **Fgf8 gone in zebrafish mutant *Ace* -- lose midbrain/hindbrain boundary as well as tectum (midbrain-derived) and cerebellum**
  - **Why do midbrain and r1 respond differently to Fgf8?**



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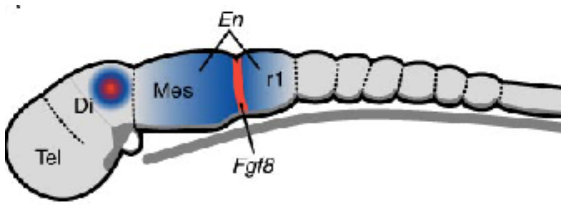
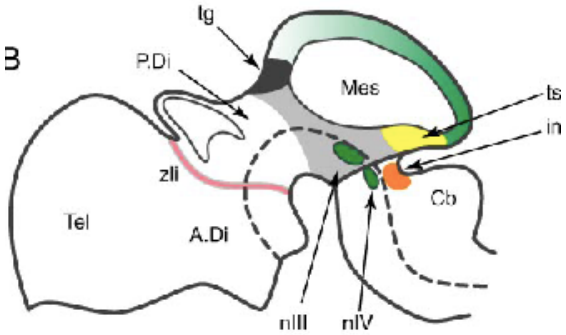
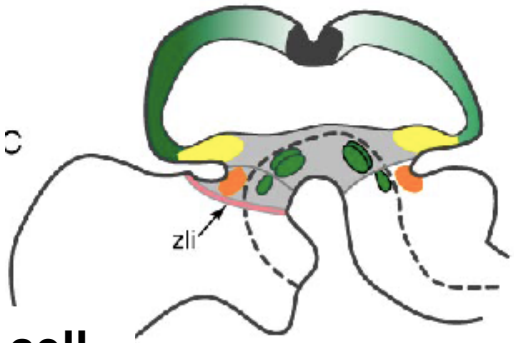
  - Why do midbrain and r1 respond differently to Fgf8?

    Could differ in competence (due to distinct cell histories) or receive additional signals







Hindbrain segmentation

- Segmentation: break tissue into repeated set of similar modules --each segment often develops as a variations on a basic theme
- Hindbrain region: after neural tube closure eight regions separated by indentations (rhombomeres) transiently appear
- Each rhombomere gives rise to cells with distinct fates

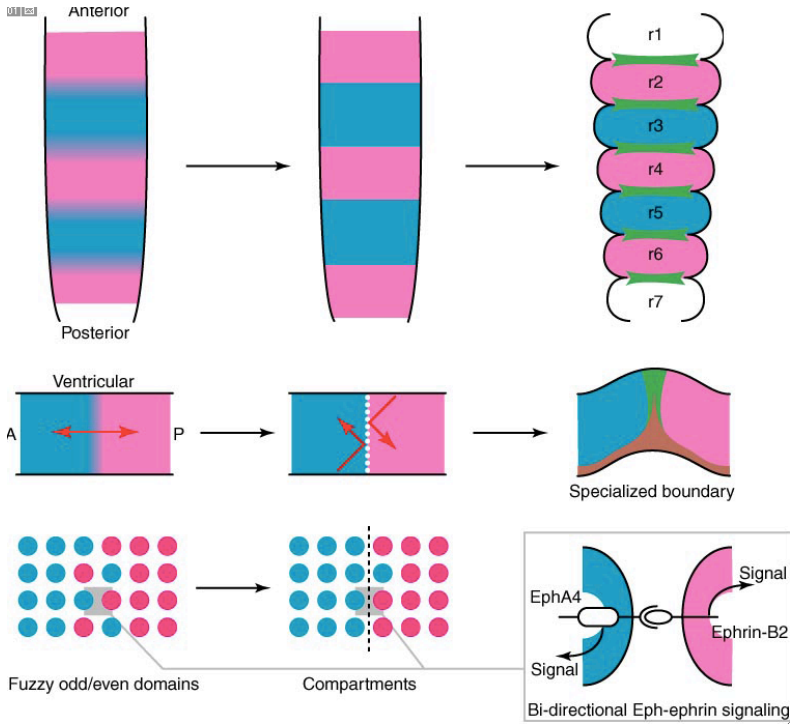
Hindbrain segmentation

- **Differential adhesion/interaction help establish boundaries and limit cell mixing facilitating each compartment's independent development**
  - **Two-segment repeat rule: odds mix with odds, evens with evens**
- **Lineage tracing experiments: inject single cells with vital dye, track progeny**
  - **Early injection --- clonal descendents appear in multiple rhombomeres**
  - **Inject after rhombomeres appear: descendents spread only within that rhombomere; at very late stages see apparent "violations" -- however, often reflects cell migrations after differentiation**

Regulators of hindbrain segmentation

- **Krox20 -- DNA-binding transcription factor**
  - **Expressed early on neural plate region that will form r3 and r5**
  - **Krox20 knckout: lose r3 and r5; r2, r4 and r6 fuse together**

- **Eph Receptor tyrosine kinases/Ephrin ligands :**
  - **Heterophilic "anti-adhesion" (repulsive) receptors --- we'll discuss these in more detail during axon guidance lectures**
  - **Eph receptors expressed in odd rhombomeres (depend on Krox20), Ephrin ligands expressed in even rhombomeres**
  - **Inhibit Eph receptors using truncated receptor --- get mixing across boundary**



Next time

- Generation of cortex
- Neuronal migration
- Neuronal stem cells