CELL 0023:
Cell Differentiation in the Developing Nervous System

Review articles:

Kicheva A, Briscoe J. Control of Tissue Development by Morphogens. Annu Rev Cell Dev Biol. 2023 Oct 16;39:91-121. doi: 10.1146/annurev-cellbio-020823-011522. Epub 2023 Jul 7. PMID: 37418774.

Sagner A, Briscoe J. Establishing neuronal diversity in the spinal cord: a time and a place. Development. 2019 Nov 25;146(22):dev182154. doi: 10.1242/dev.182154. PMID: 31767567.

CNS specification involves:

1) Induction of ectoderm into neural tissue
2) Patterning along the anterior-posterior axis
3) Patterning along the dorso-ventral axis

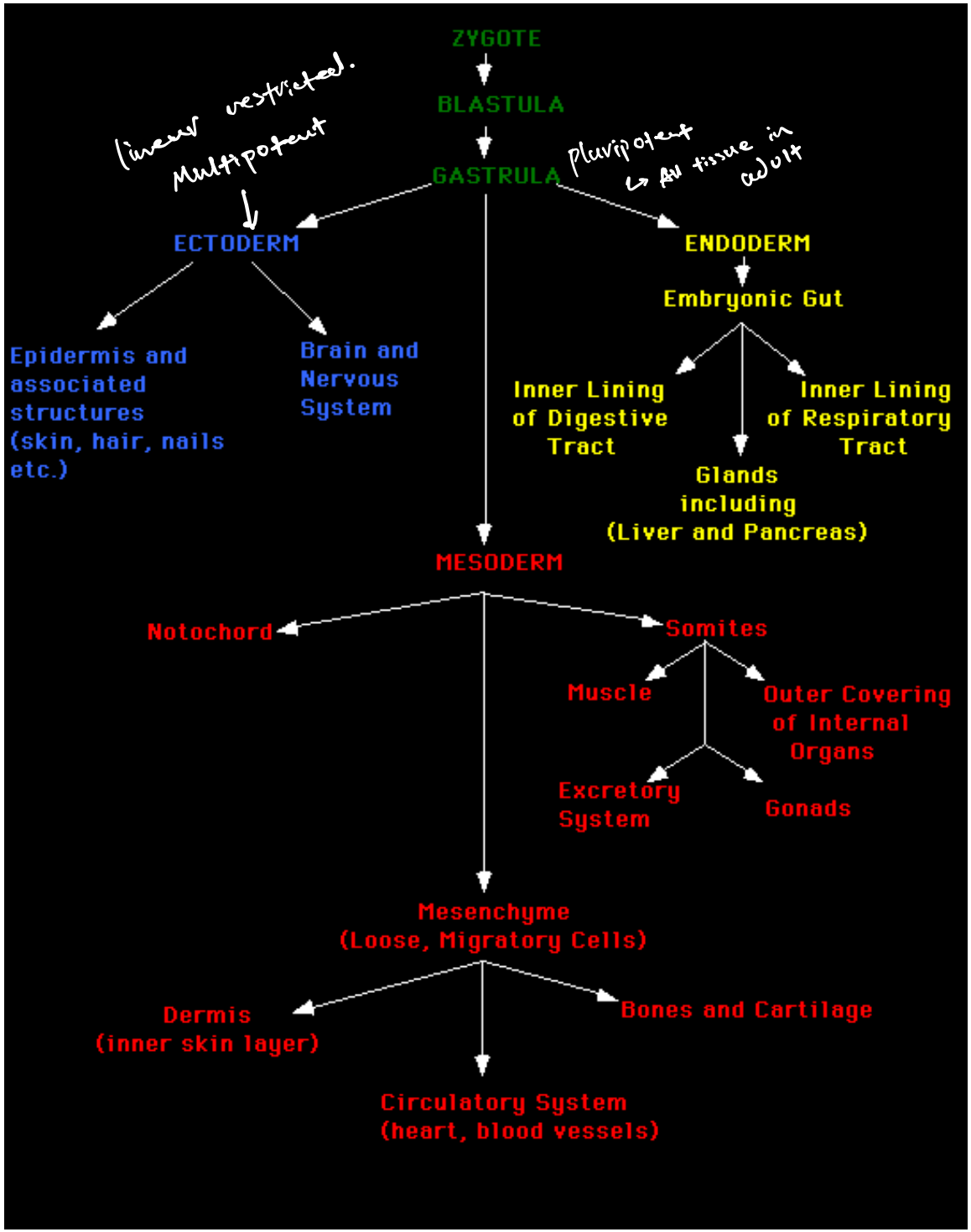
Cells become more specialized – this restricts the types of cells they can generate

(multipotent not totipotent)

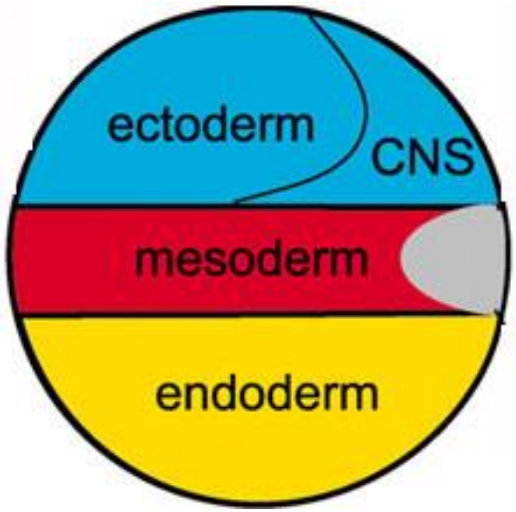
<u>Blastula:</u>
Ball of unspecialized cells

<u>Gastrulation:</u>
Early stage in differentiation to form 3 layers

<u>3 Germ layers:</u>
Ectoderm
Mesoderm
Endoderm



Induction of Ectoderm into Neural Tissue



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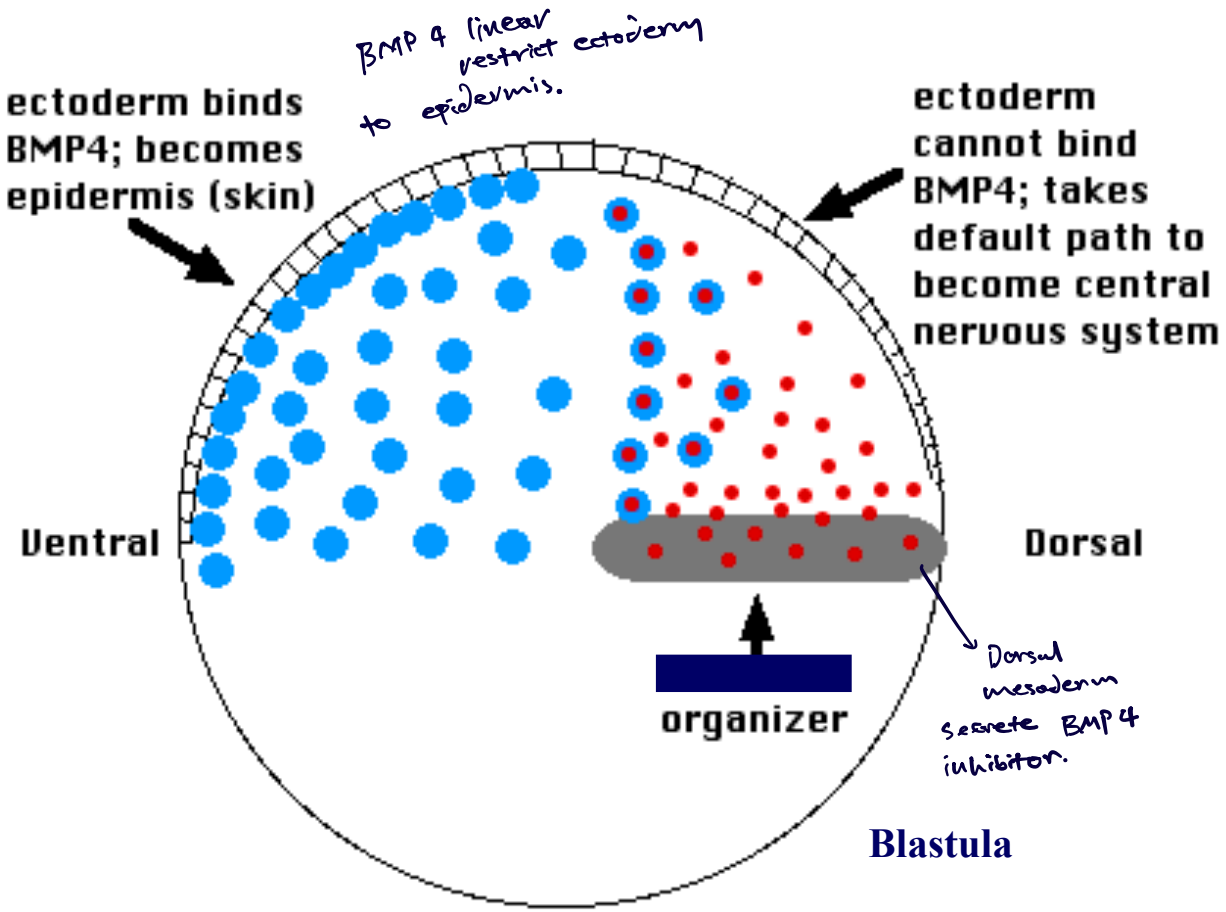

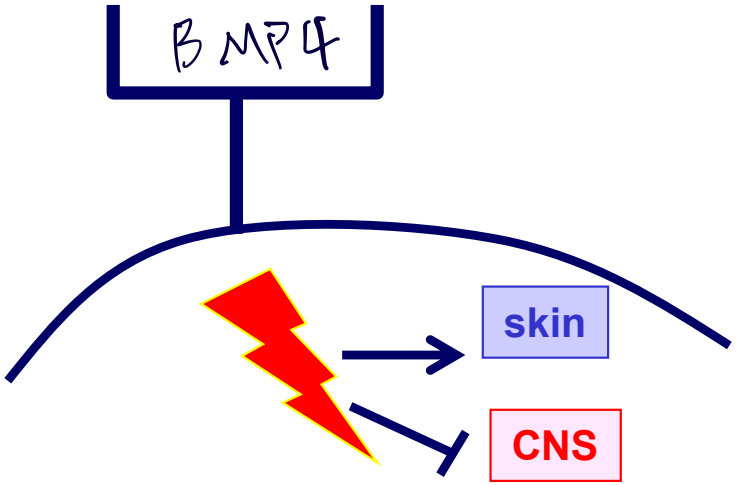

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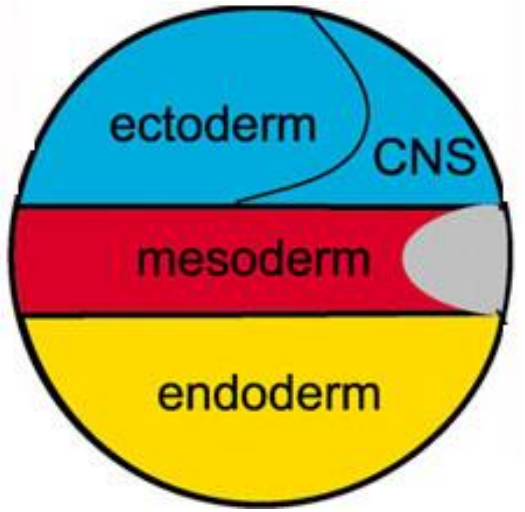
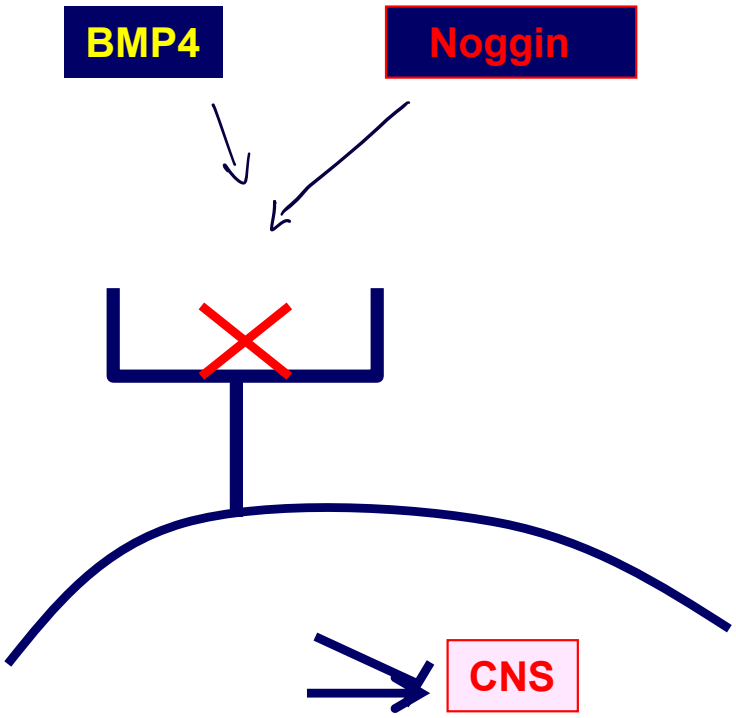
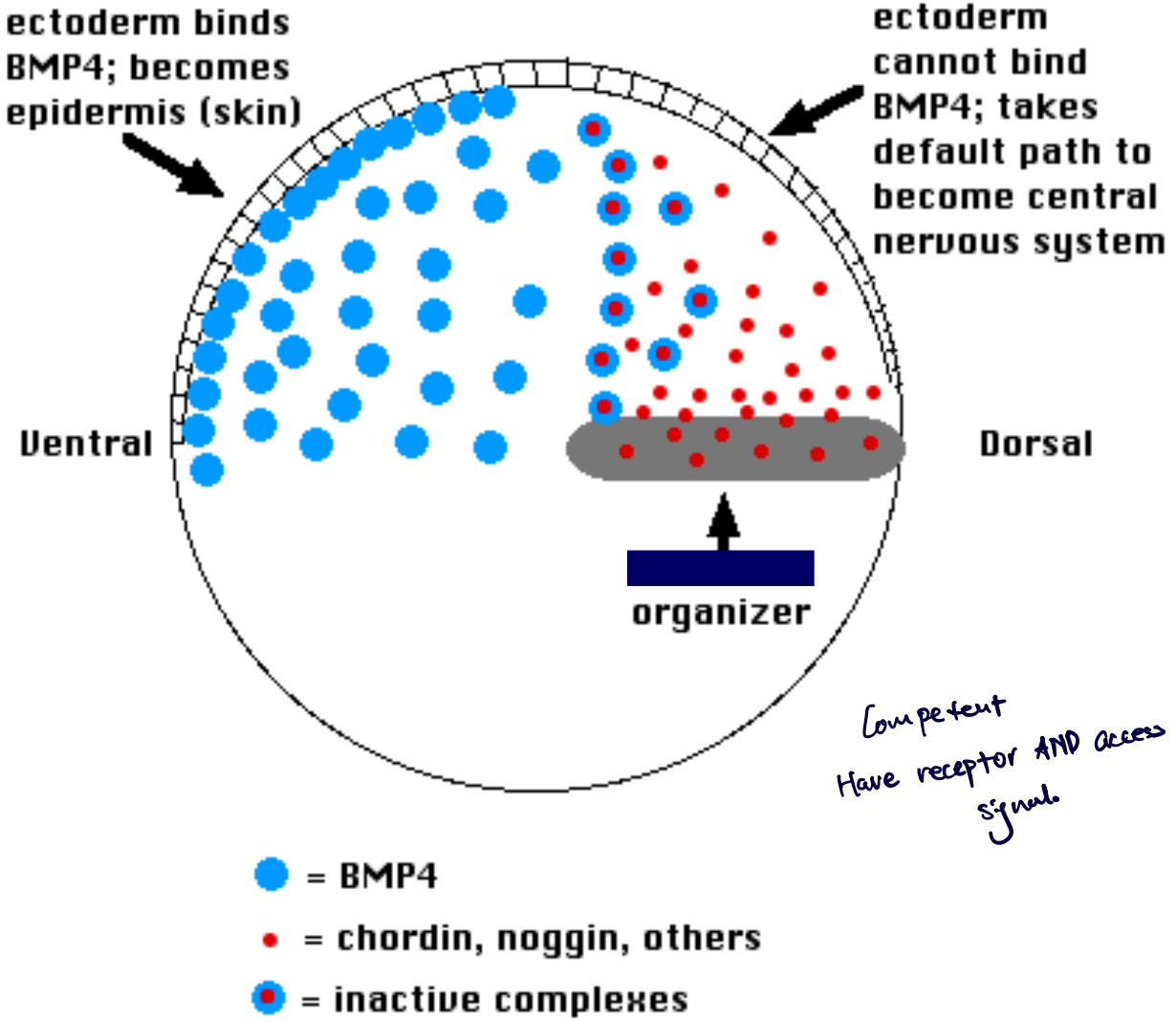
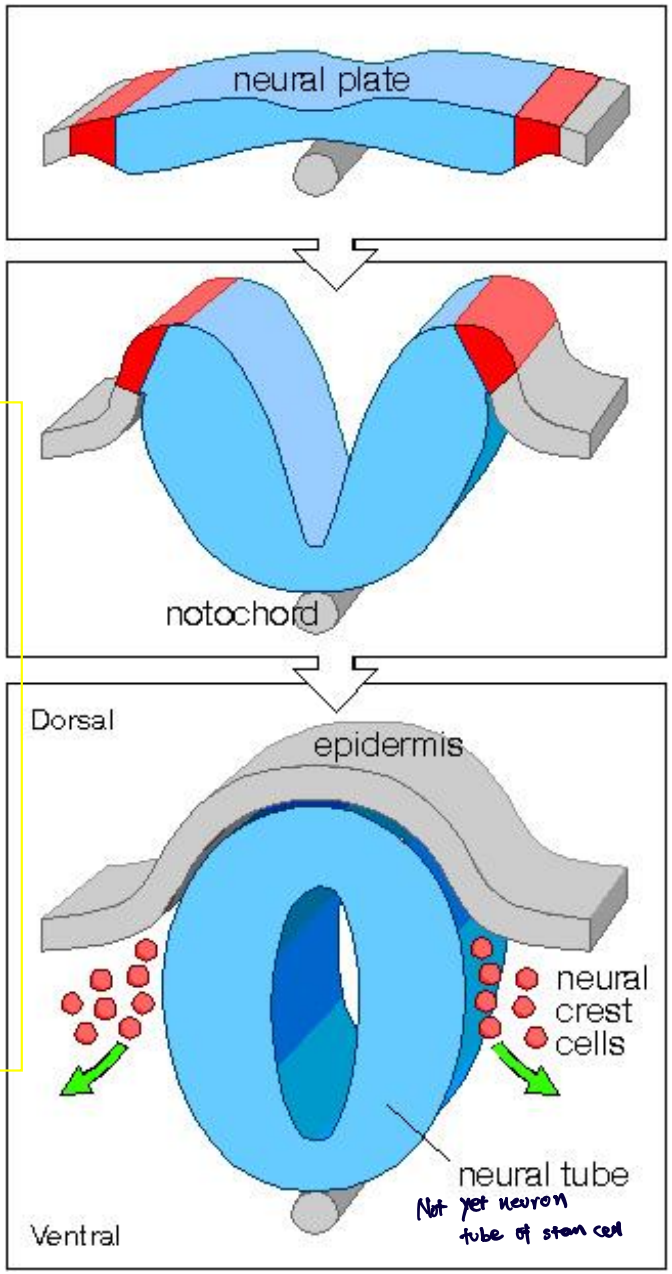




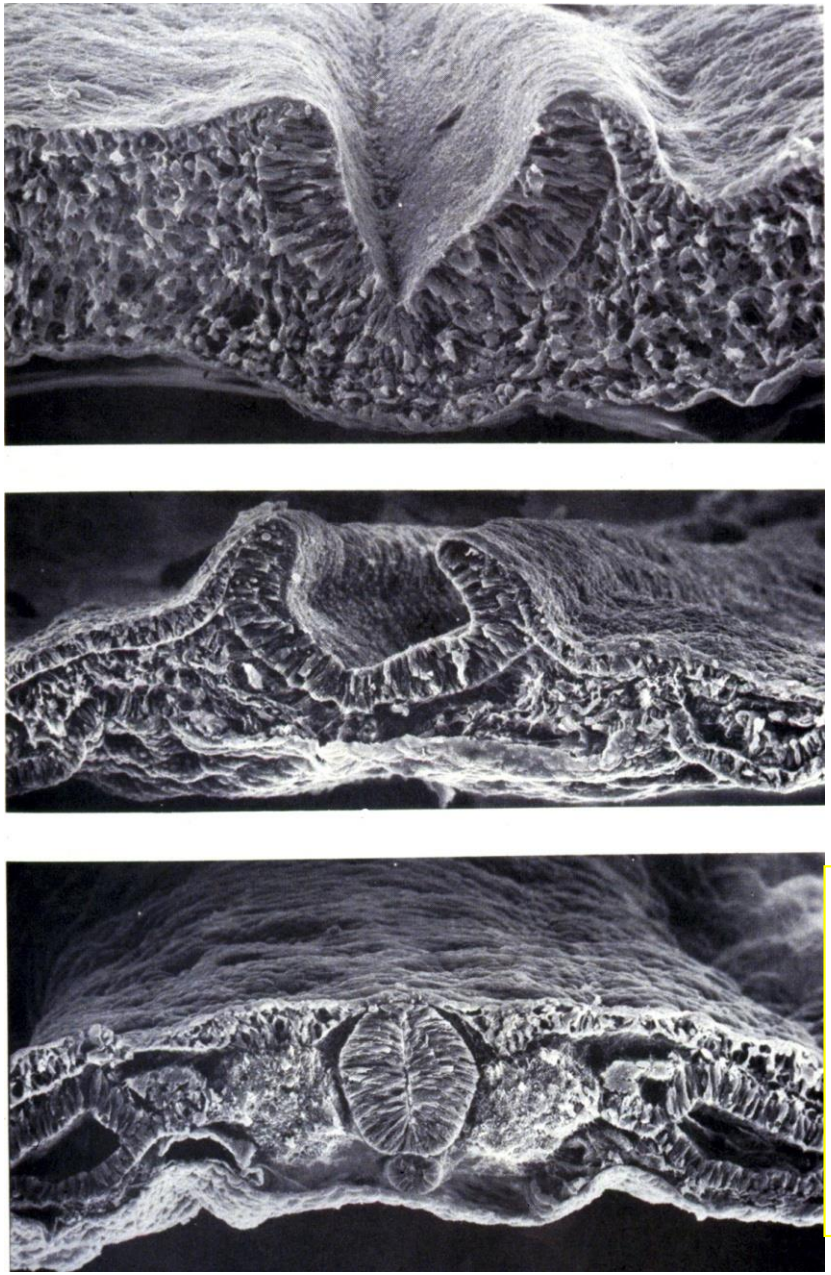


Competent
Have receptor AND access to signal

Initial development of the neural tube



Scanning electron micrographs



Neural crest cells form the peripheral nervous system including most of the sensory and all of the sympathetic ganglia and schwann cells

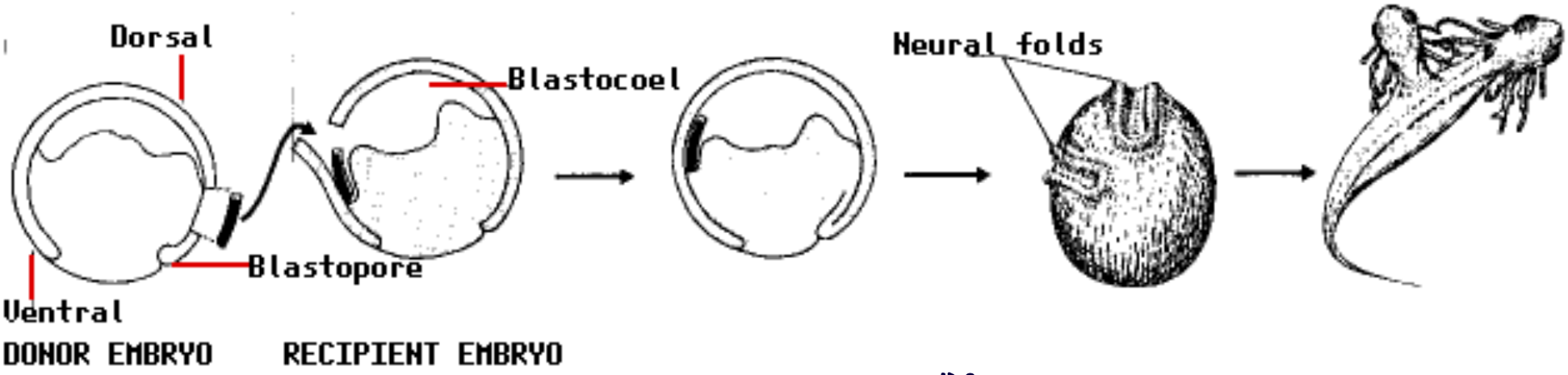
The neural tube will form the brain and spinal cord

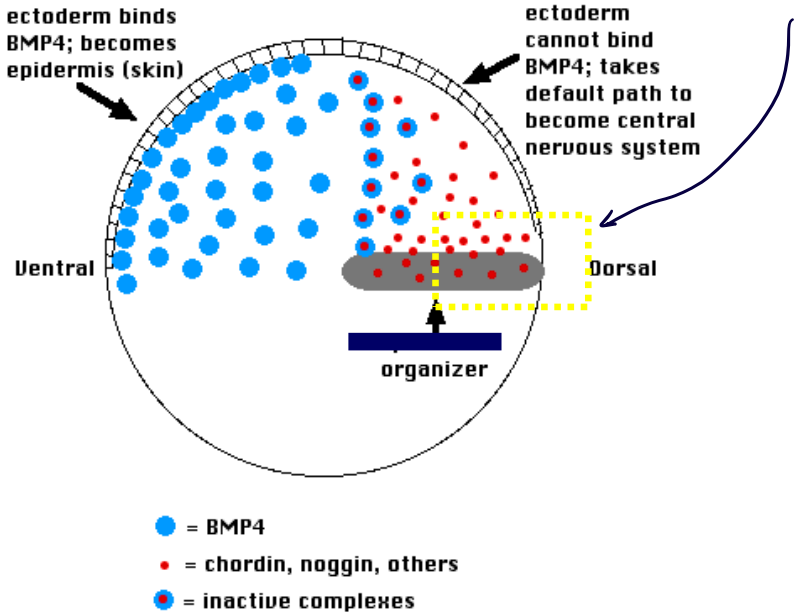
Signaling from the organizer (notochord) causes ectodermal cells to thicken and roll up

CNS specification involves:

1) Induction of ectoderm into neural tissue
2) Patterning along the anterior-posterior axis
3) Patterning along the Dorso-ventral axis

Anterior-Posterior Patterning in the Blastula



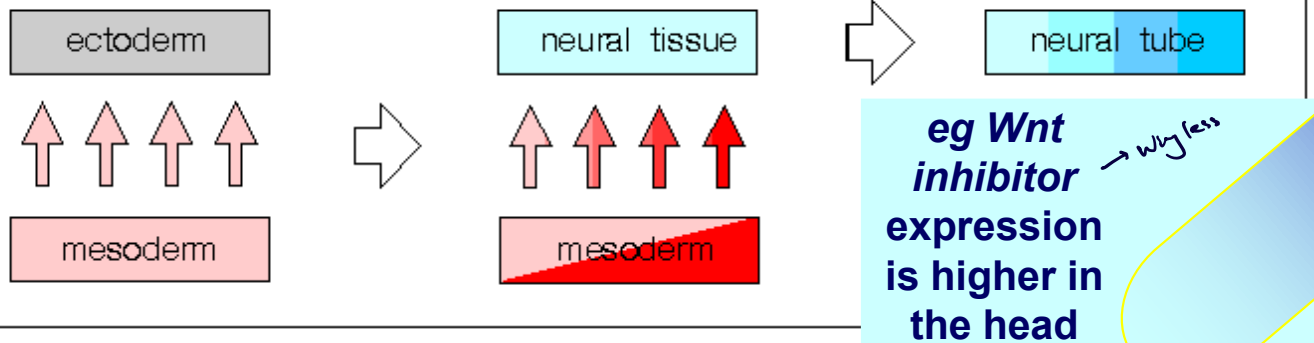


AP axis start at the organiser

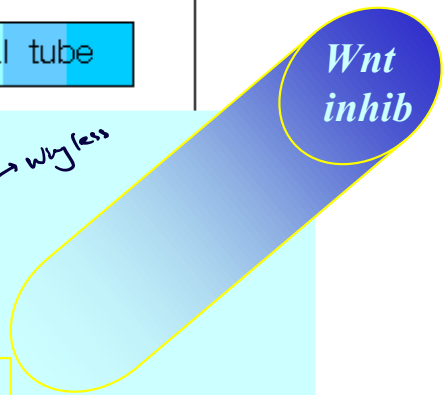
The organizer (primitive node) is a very powerful signaling centre - if organizer tissue from the head end of a xenopus tadpole is transplanted into another tadpole a second head develops!

Models of AP specification

1) Signalling by a morphogen gradient along the length of the organizer



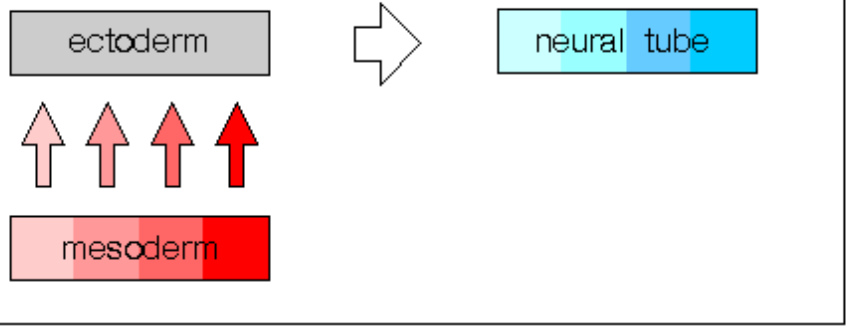
Head / anterior



eg Wnt inhibitor **expression is higher in the head**

Tail / posterior

2) Different neural inducers expressed at different levels of the organizer



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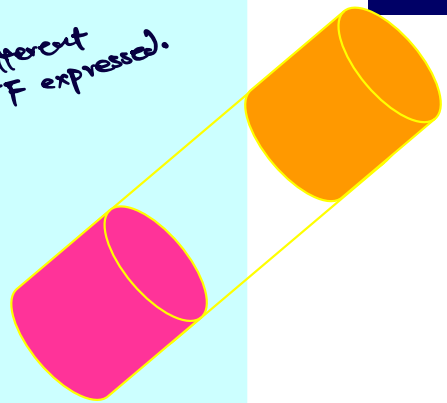
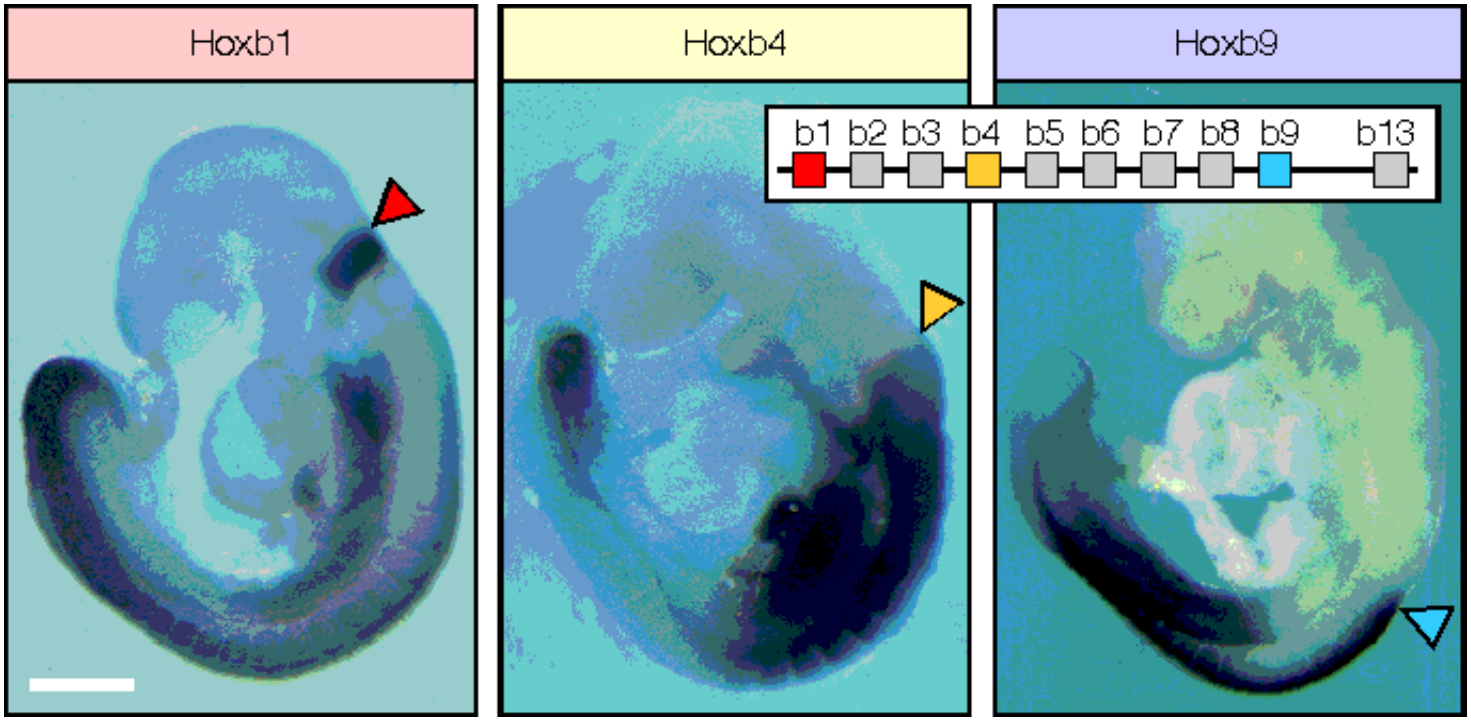


eg Lim-1 **is expressed in the head and** ***Brachyury*** **is expressed in the tail**

Tail / posterior

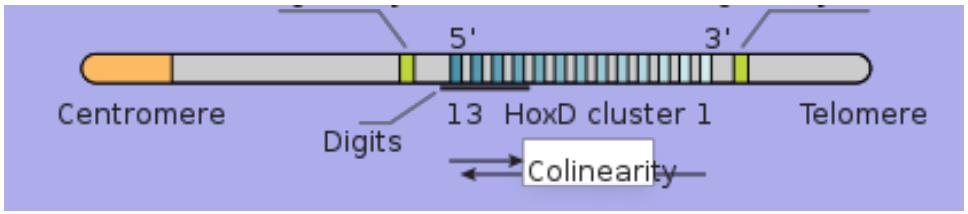
Mesoderm = organizer

Hox gene expression in the AP axis of the mouse



large family of clustered genes.

Co-linearity: location on chromosome correspond to position on A-P axis



- **The expression of a Hox gene along the AP axis is linked to its chromosomal position**
- **Combinatorial expression of Hox gene expression codes for location in the AP axis**

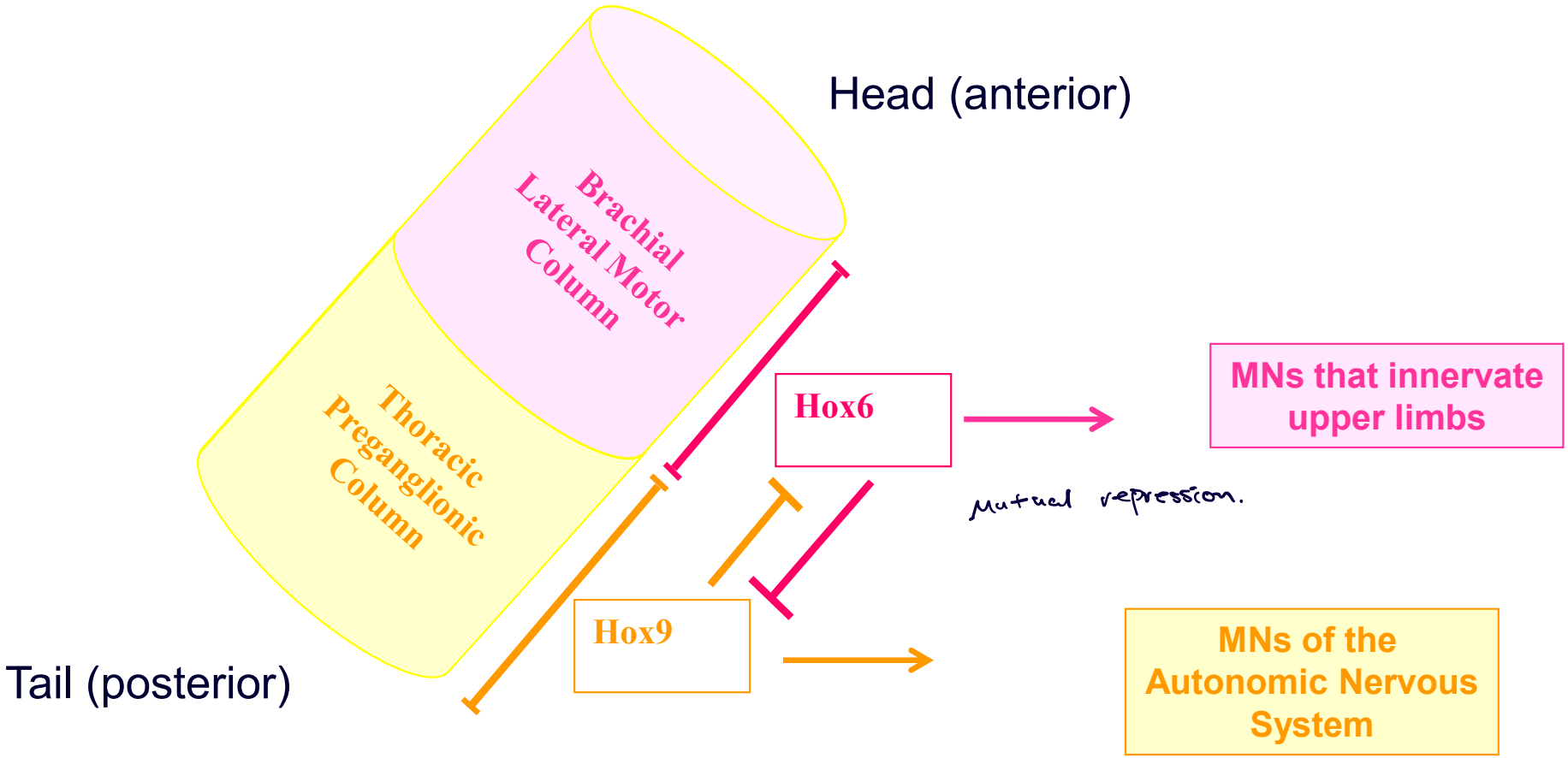
A-P Specification of Motor Neurons

- Motor neurons (MN) are the exclusive action link between the nervous system and motor output
- MN number, identity and connectivity to match the peripheral target which varies at each level of the spinal cord
- MN cell bodies are organized into motor columns according to broad projection territories such as upper limbs (brachial lateral motor column) or the autonomic nervous system (preganglionic column)– the relevant column forms only at the appropriate segmental level
- Within the motor columns groups of MNs projecting to individual muscles are clustered into MN pools
- The combinatorial expression patterns of Hox genes at the different AP levels of the spinal cord plays a key role in assigning both columnar and MN pool fate to the developing MNs

Different classes of genes classify different MN population

Anterior-Posterior Patterning of the Spinal Cord Specifies Motor Column Identity

Hox6 or Hox9 are sufficient to transform columnar identity



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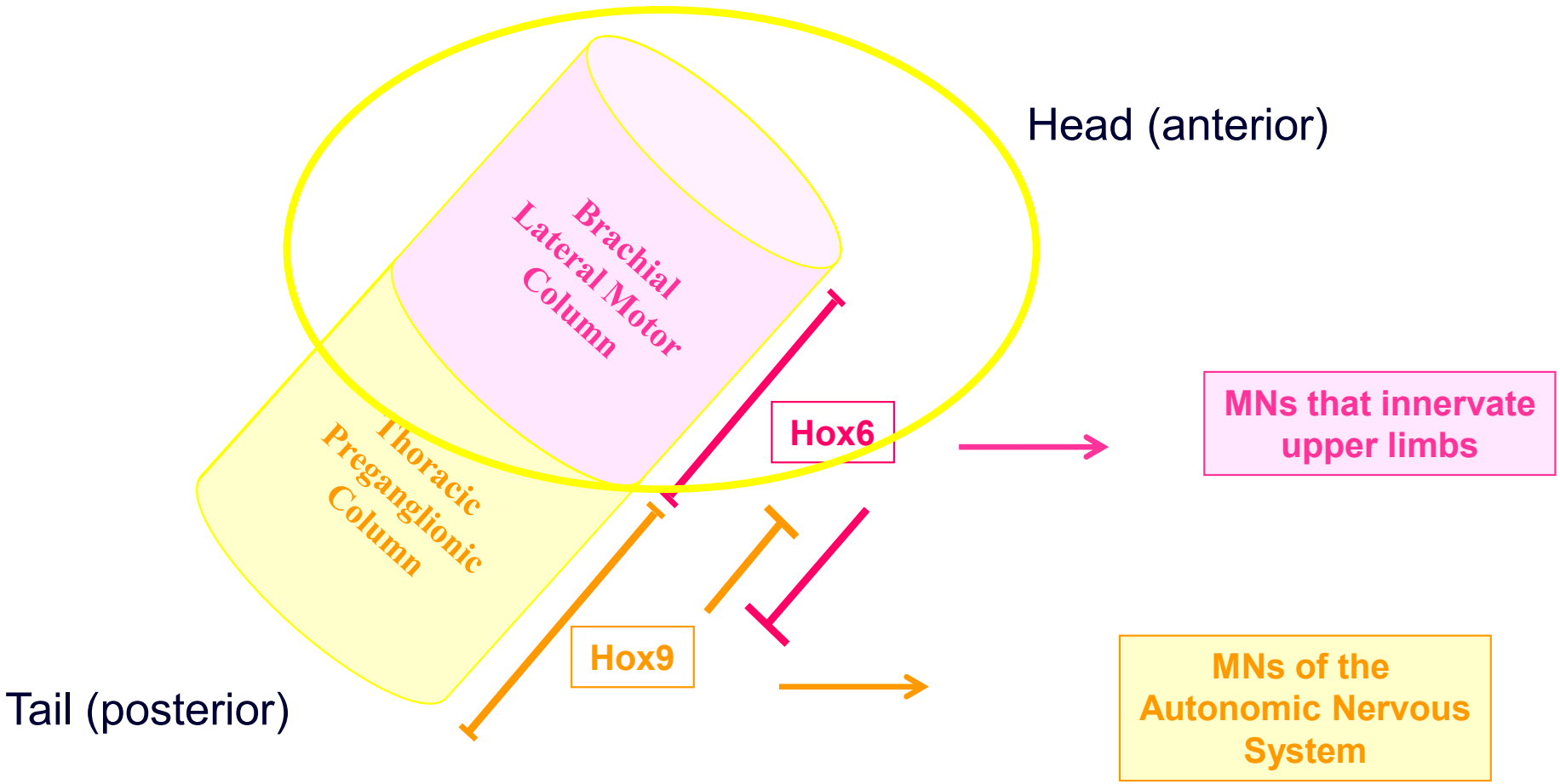

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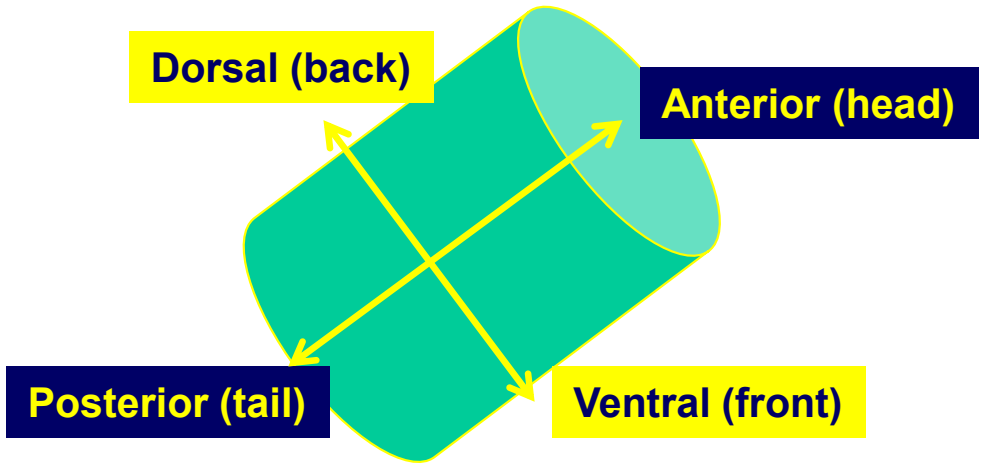
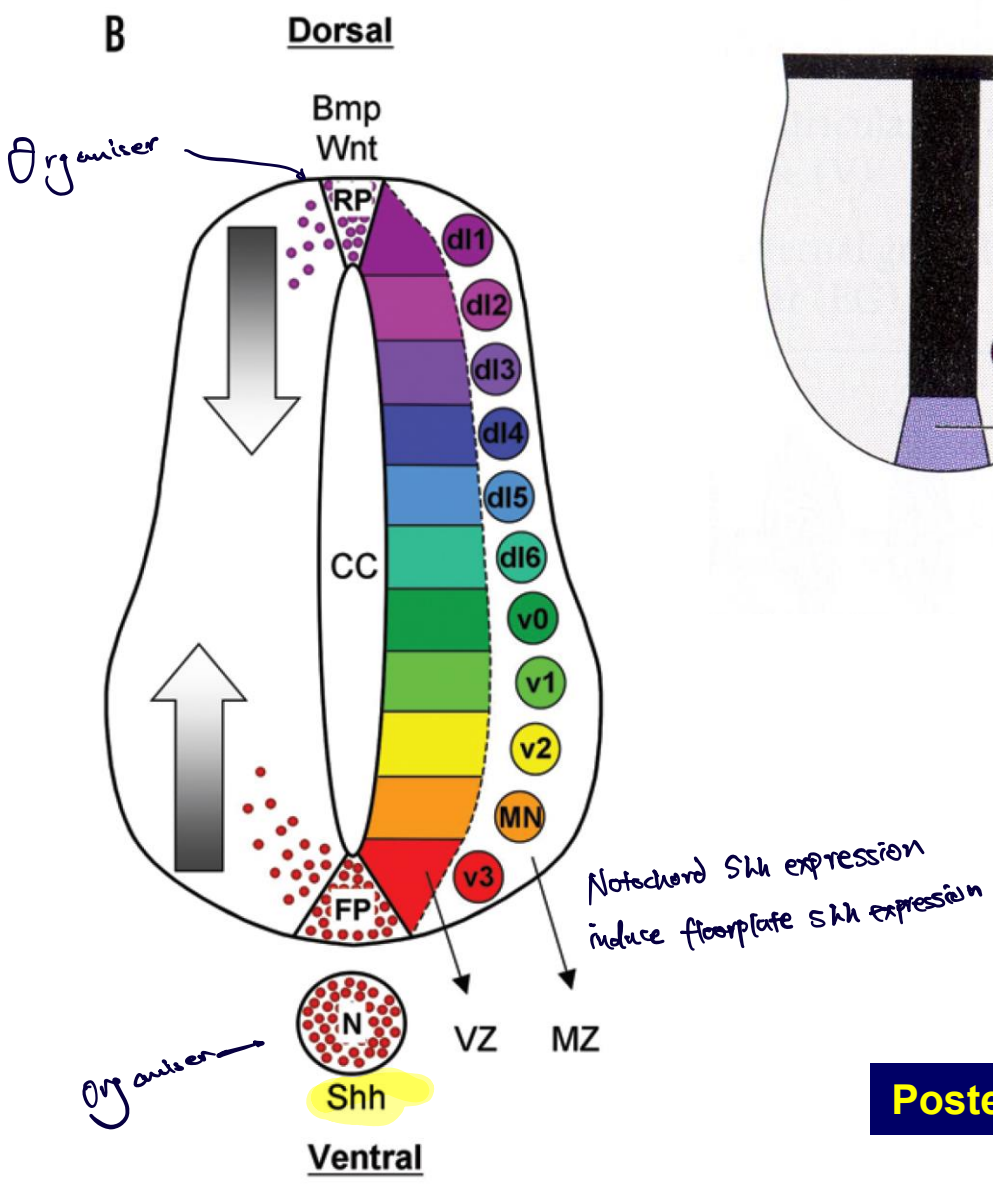
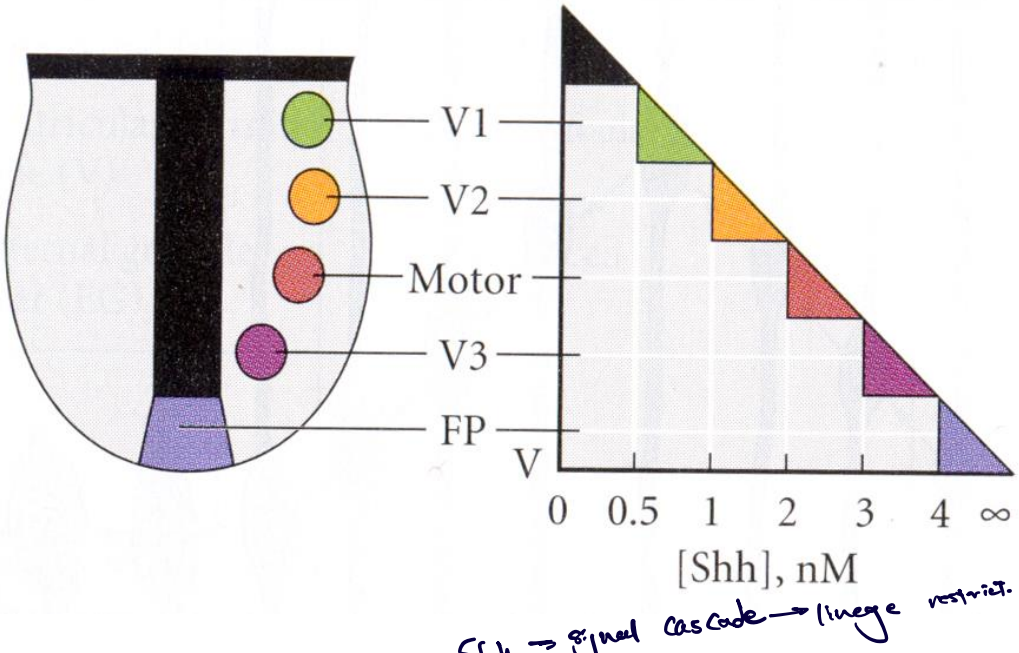
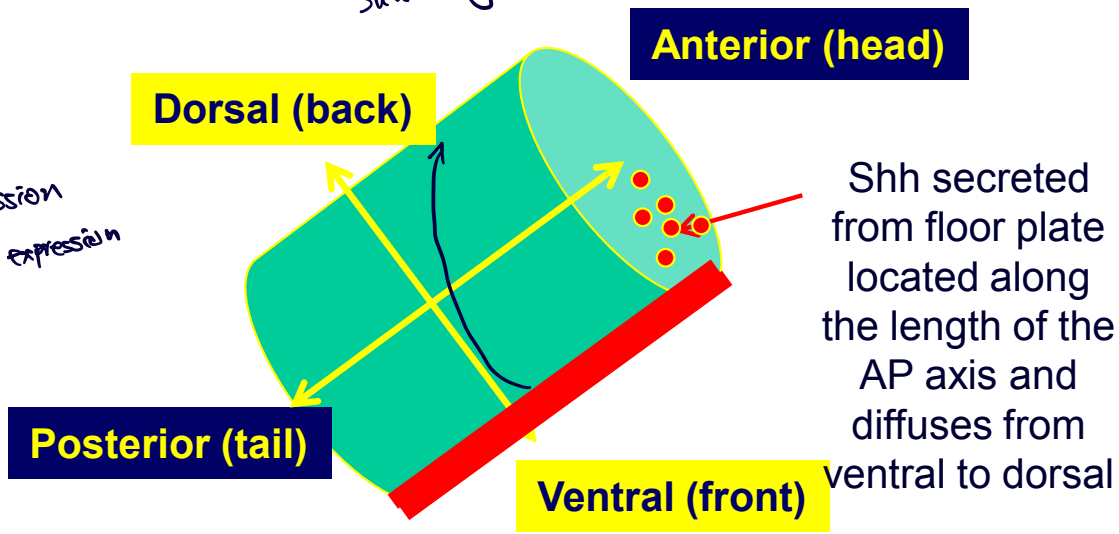


Different cell types are generated at different dorsal ventral locations within the spinal cord







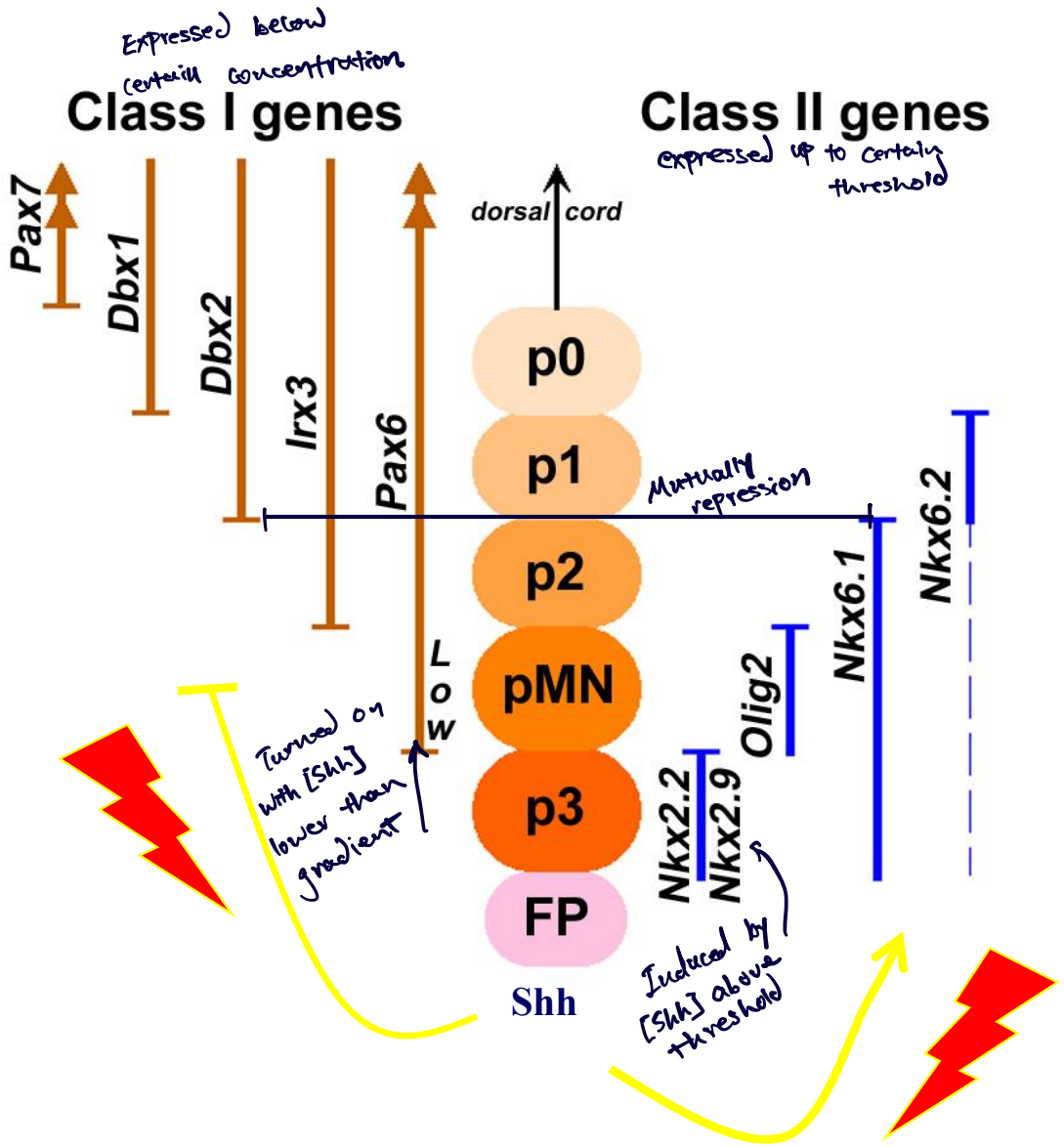
Transduction of continuous gradient → Discrete layers.

How does Shh signaling translate to different types of neurons being generated?

- Different class I transcription factors are repressed by different concentrations of Shh – preventing their expression in the ventral spinal cord

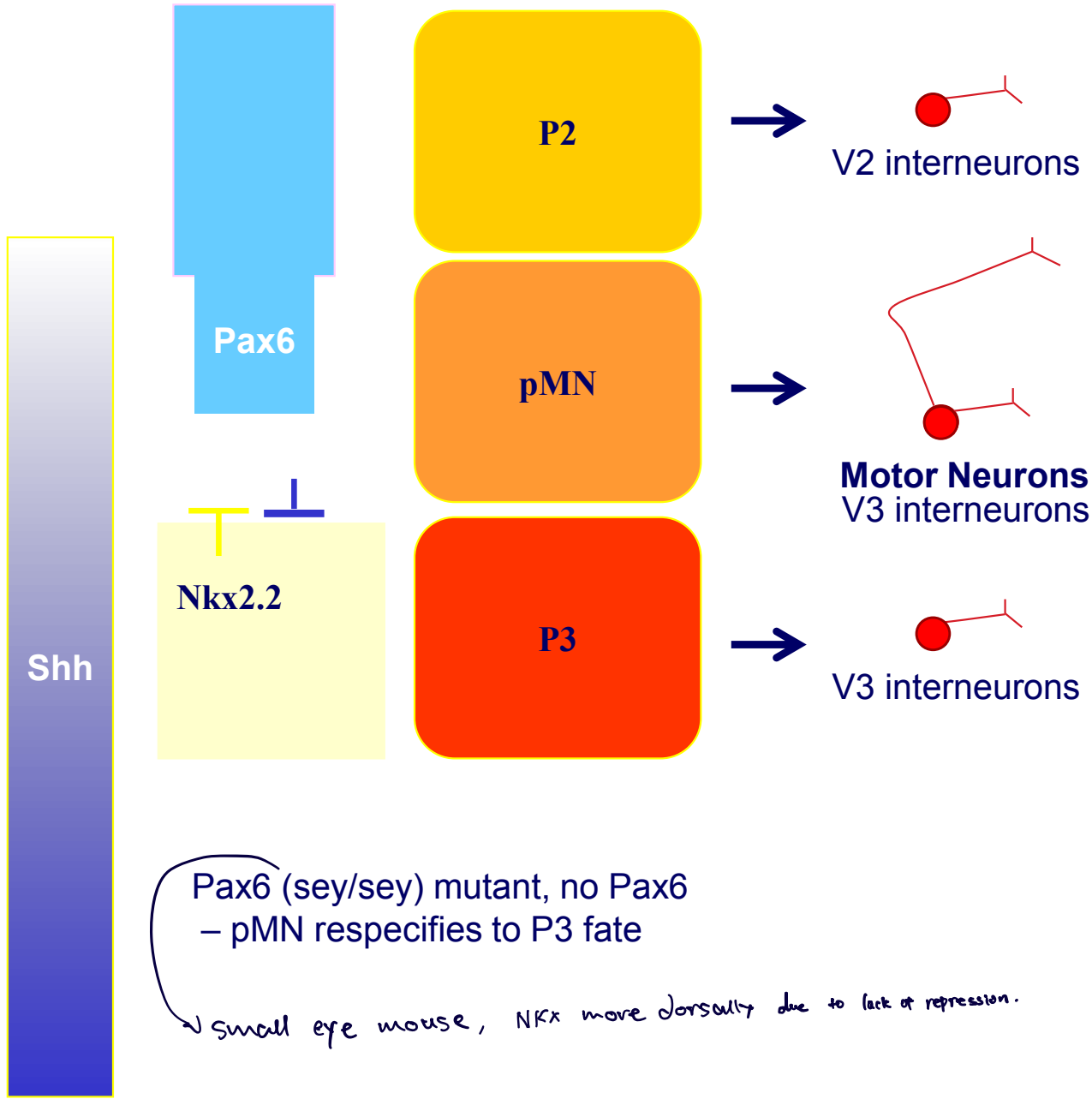
- Different class II transcription factors are induced by Shh at different concentrations – turning on their expression in the ventral spinal cord

- Many of these transcription factors set up boundaries by cross repression



Cross Repression Enforces Boundaries of Each Neuroepithelial Domain to Specify Neuron Type

Transcription Factors enhance the expression of genes necessary for the "chosen" cell fate while suppressing all of the alternative cell fates



Pax6 (sey/sey) mutant, no Pax6
– pMN respecifies to P3 fate

small eye mouse, NKx move dorsally due to lack of repression.

Cross Repression Enforces Boundaries of Each Neuroepithelial Domain to Specify Neuron Type

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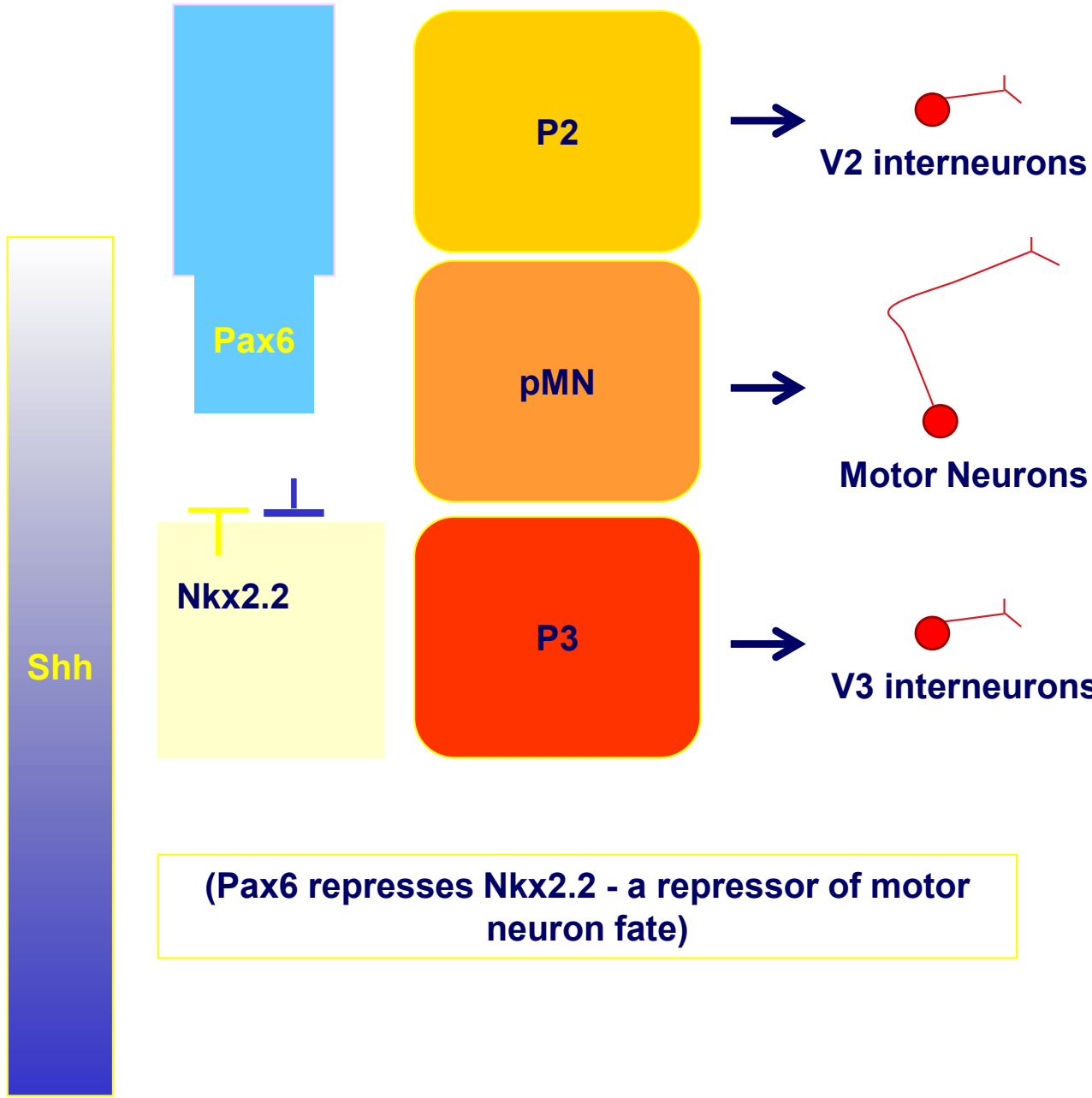
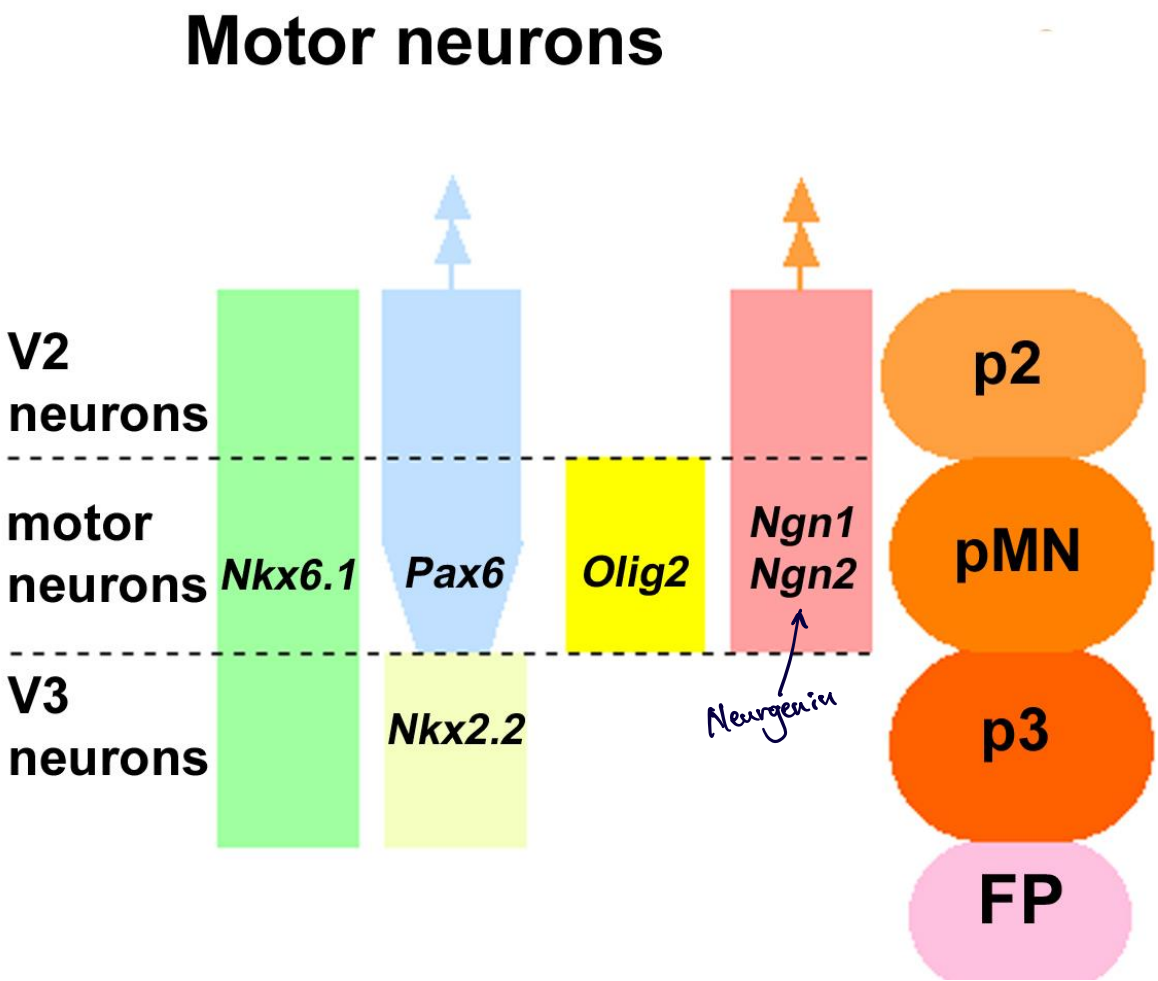
Nkx2.2 Prevent expression of Pax6.



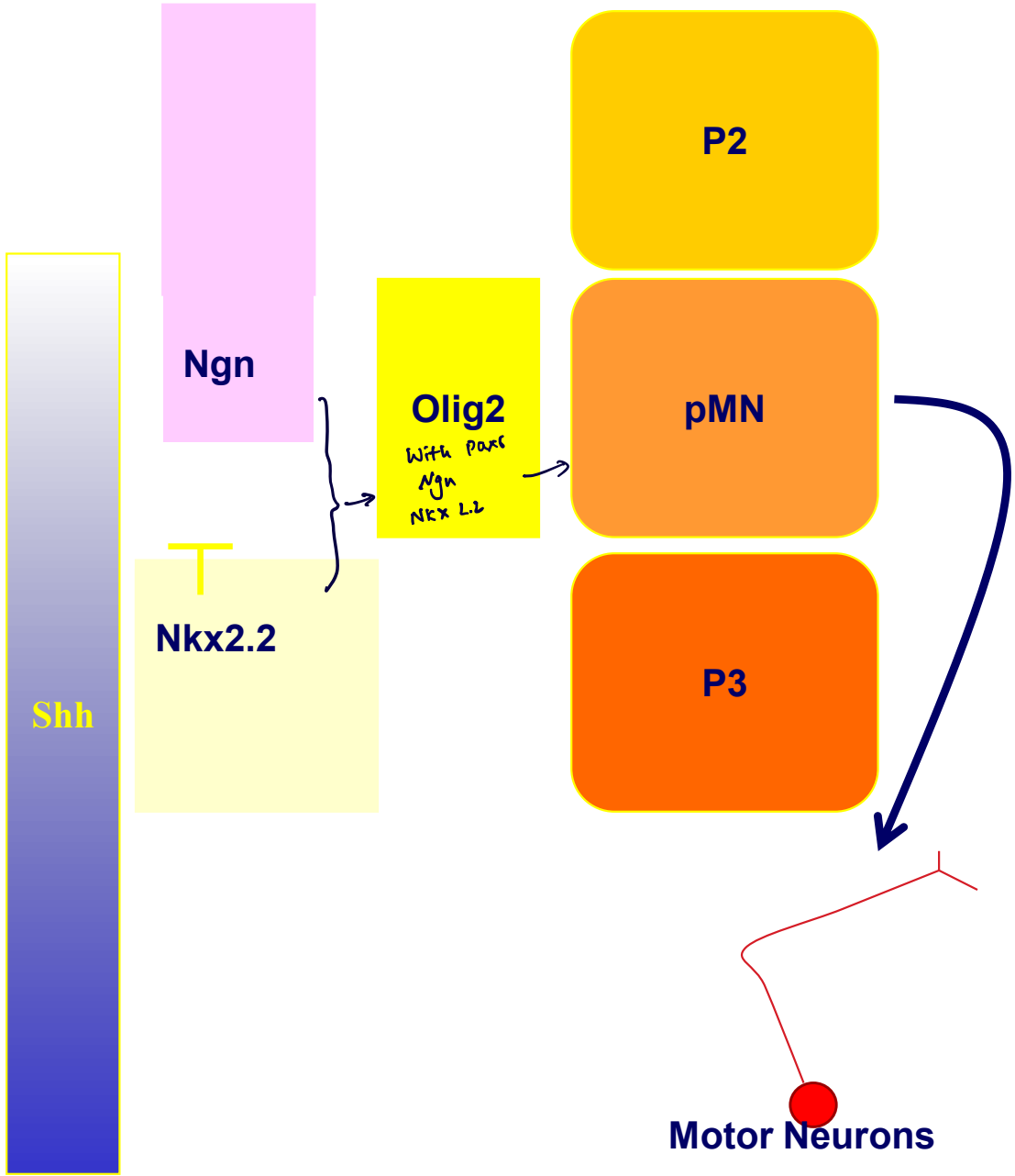
(Pax6 represses Nkx2.2 - a repressor of motor neuron fate)

Combinations of different transcription factors at any single level of the spinal cord will determine which types of neurons are generated



Motor Neuron specification requires the transcription factors Olig2 and neurogenin

Transcription Factors
enhance the expression of genes necessary for the "chosen" cell fate while suppressing all of the alternative cell fates



Specification of glial cells

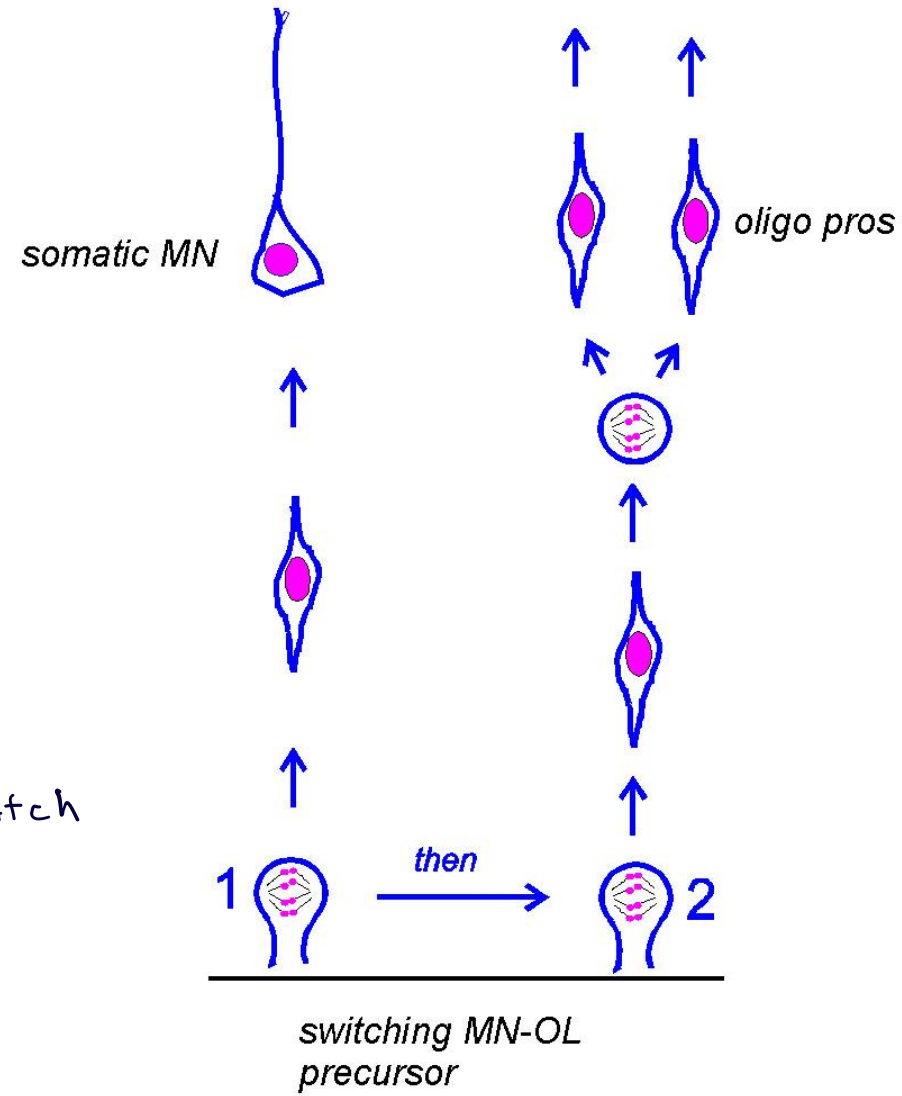
- Glial cells are born after neurons have been born
- Glial cells (unlike neurons) are able to proliferate after they have been born
- There are two major types of glial cells in the CNS astrocytes and oligodendrocytes
- We are going to confine ourselves to oligodendrogenesis - the generation of the myelinating cells of the CNS
- The first born cells are known as oligodendrocyte progenitors - these cells will eventually differentiate into mature myelinating cells

Oligodendrocyte progenitors arise after all the motor neurons have been born and from the same part of the spinal cord.

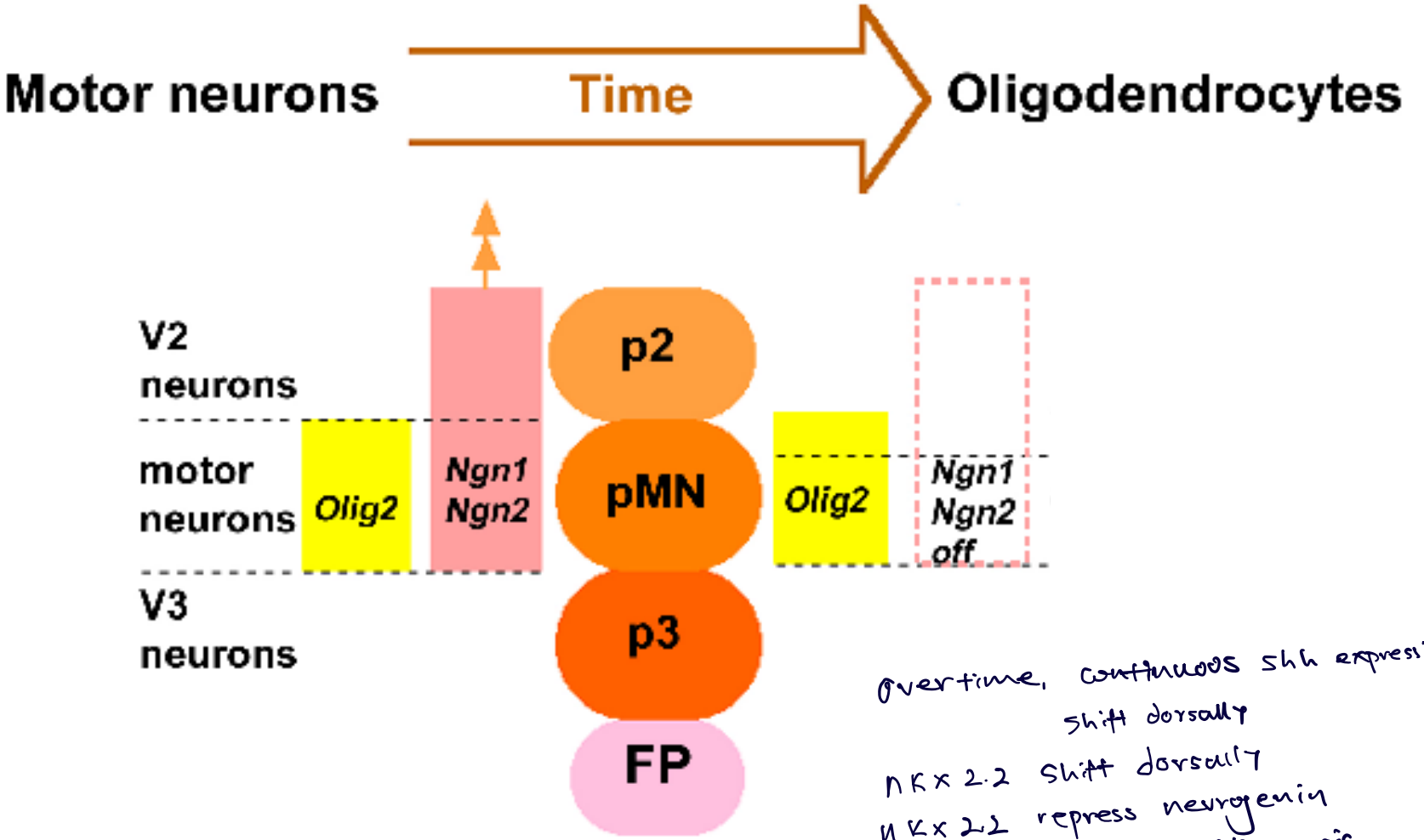
olig2 critical for oligodendrocyte.

How do the cells in the pMN domain switch from making motor neurons to making oligodendrocyte progenitors?

switch



Oligodendrocyte specification requires Olig2 and the downregulation of neurogenin

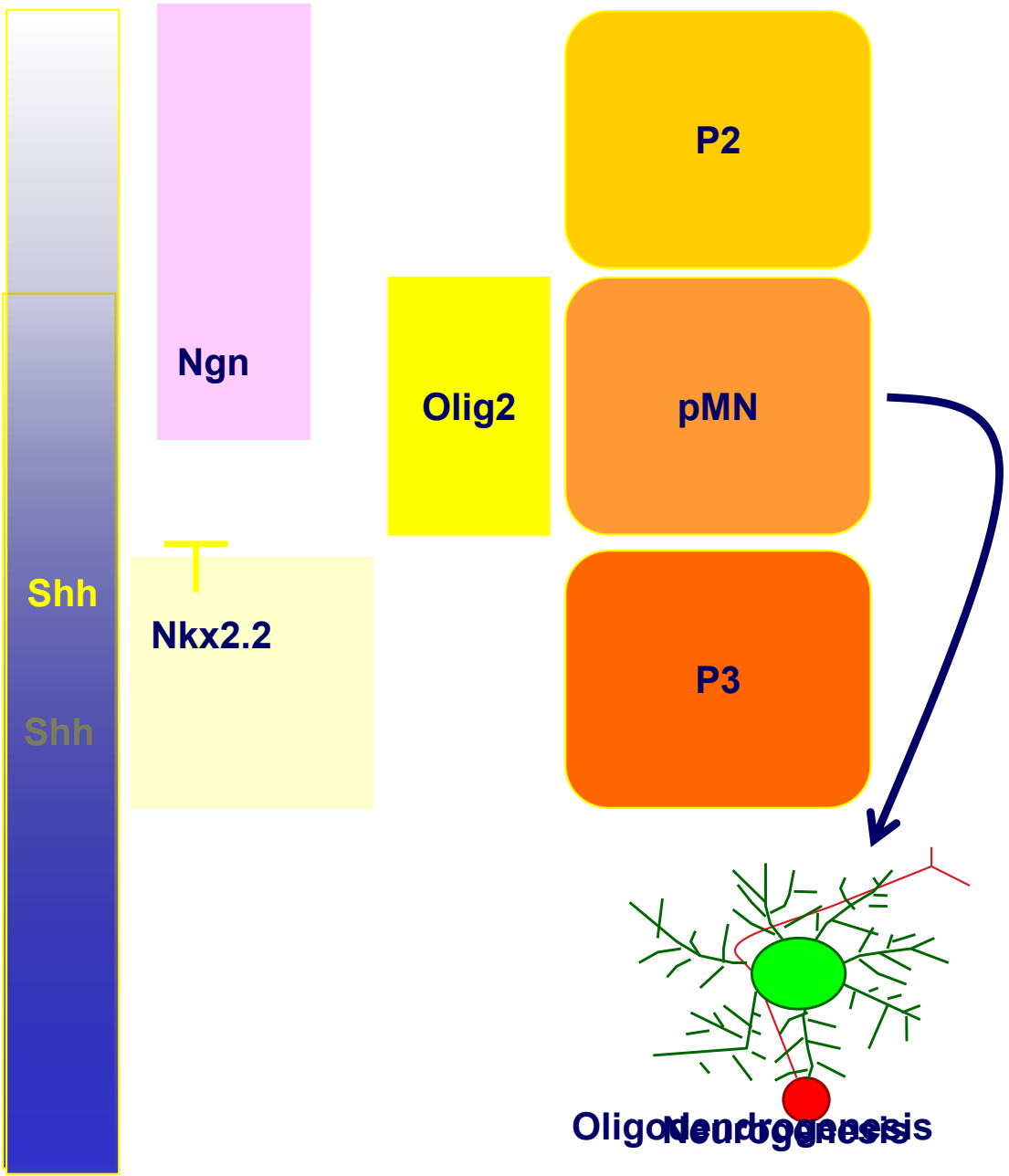


overtime, continuous shh expression
shift dorsally
NKX 2.2 shift dorsally
NKX 2.2 repress neurogenin
NKX 2.2 + olig2 → gliogenesis

Oligodendrocyte specification requires Olig2 and the downregulation of neurogenin

Transcription Factors enhance the expression of genes necessary for the "chosen" cell fate while suppressing all of the alternative cell fates

Suggests that timing of Shh exposure is also an important determinant



Role of Shh

- Best known for its role in patterning and neuronal subtype specification
- Can influence cell proliferation
- Can promote cell survival

  – Three classic growth factor functions!

Interconnected Mechanisms of Differentiation

- Environmental cues eg growth factors regulate progenitor cell proliferation, lineage commitment and survival
- Differentiation requires transcription factors to turn on synthesis of proteins for the selected cell fate and suppress synthesis of proteins associated with other cell fates
- Apoptosis is central to regulating the number of cells and the type of differentiated cells that survive – cells require a signal to survive

Summary

- All the neurons and glial found in the adult CNS develop from a single layer of neural stem cells forming the neural tube.
- Hox genes provide positional information in the anterior-posterior axis of the neural tube / CNS
- A gradient of Shh protein from the notochord and floor plate provides positional information to neural stem cells along the dorso ventral axis of the neural tube / CNS
- Shh concentration signals the repression or induction of Class I and Class II transcription factors respectively

Summary

- Interactions between Class I and class II transcription factors specify domains along the D/V axis
- The combinations of Class I class II genes that are expressed at any particular level of the cord will determine neuronal identity
- Generation of motor neurons in the pMN domain requires expression of neurogenin and Olig2 transcription factors.
- The switch in the pMN domain from neurons to oligodendrocytes requires the down regulation of neurogenin (pro-neural) and the continued expression of Olig2

Review Article- a bit complex but covers a lot

Neuron differentiation

Patterning in the CNS

During vertebrate development, pluripotent cells from the embryo becomes linear restricted as they separate into three layers. The three germ layers are multipotent. They are competent to respond to various signals and become many cell types.

Specification of the neural tissue from the ectoderm:

- BMP can bind to ectoderm tissues to induce epithelial fate, inhibit neuronal fate
- CNF (Chordin, Noggin, Follistatin) can bind to the receptor, preventing BMP binding.

Anterior-Posterior tissue patterning:

- Morphogen gradient model: a morphogen is expressed at different concentration along the A-P axis
- Neural inducer model: differet inducers expressed at different parts along the A-P axis
- Hox expression: Co-linear expression of morphogenic patterning gene along the A-P axis, corresponding to position on the chromosome

A-P patterning of motor neurons

- Unique combination of Hox gene specifies the identity of a particular spinal segment along the A-P axis (e.g. brachial vs. lumbar)
- E.g. within the spinal cord, anterior Hox-6 and posterior Hox-9 exhibit mutual exclusion. Anterior spinal cord express brachial lateral motor column while posterior spinal cord produce thoracic preganglionic column.
    - **Experiment**: Expression of Hox9 at the anterior end of the neural tube induce autonomic fate adoption
- Transcription factor activate downstream pathways help specify post-mitotic fates

Dorsoventral patterning of the spinal cord.

- On the ventral side, the notochord act as the organiser, induce the floorplate to become the organiser, secrete sonic hedgehog (Shh)
- Floor plate + notochord establish a gradient of Shh ventrodorsally, counteracts with the dorsoventral gradient of the roof plate expressing Wnt and BMP, lineage restrict the stem cell fates along the D-V axis
- Different transcription factors are turned on/off when Shh exceed a certain threshold concentration
    - E.g. Class I TF such as Nkx2.2 are induced by Shh, class II TF such as Pax6 is repressed by Shh
    - The two classes of TF mutually repress each other and eatsblish boundaries
- The combinatorial TF expression at different segments specifies cell fates
    - E.g. P2, pMN and P3 are progenitor pools having high to low Shh concentration, giving rise to corresponding V2 interneuron, motor neurons and V3 interneurons
    - In small eye mutant mice which lacks Pax6 transcription factor, Nkx2.2 will be expressed more dorsally, its pMN wil be converted to P3

Cell type specification within the same A-P D-V axis - temporal regulation

- In the pMN progenitor pool, some neuron progenitors become oligodendrocytes as development progress
- This is due to increasing Shh concentration, shifting the gradient dorsally, a shift in the TF combination (absence of neurogenin) leads to differentiation of MN into oligodendrocyte.