Cell Fate II
(Determination & Differentiation)

Steven McLoon
Department of Neuroscience
University of Minnesota

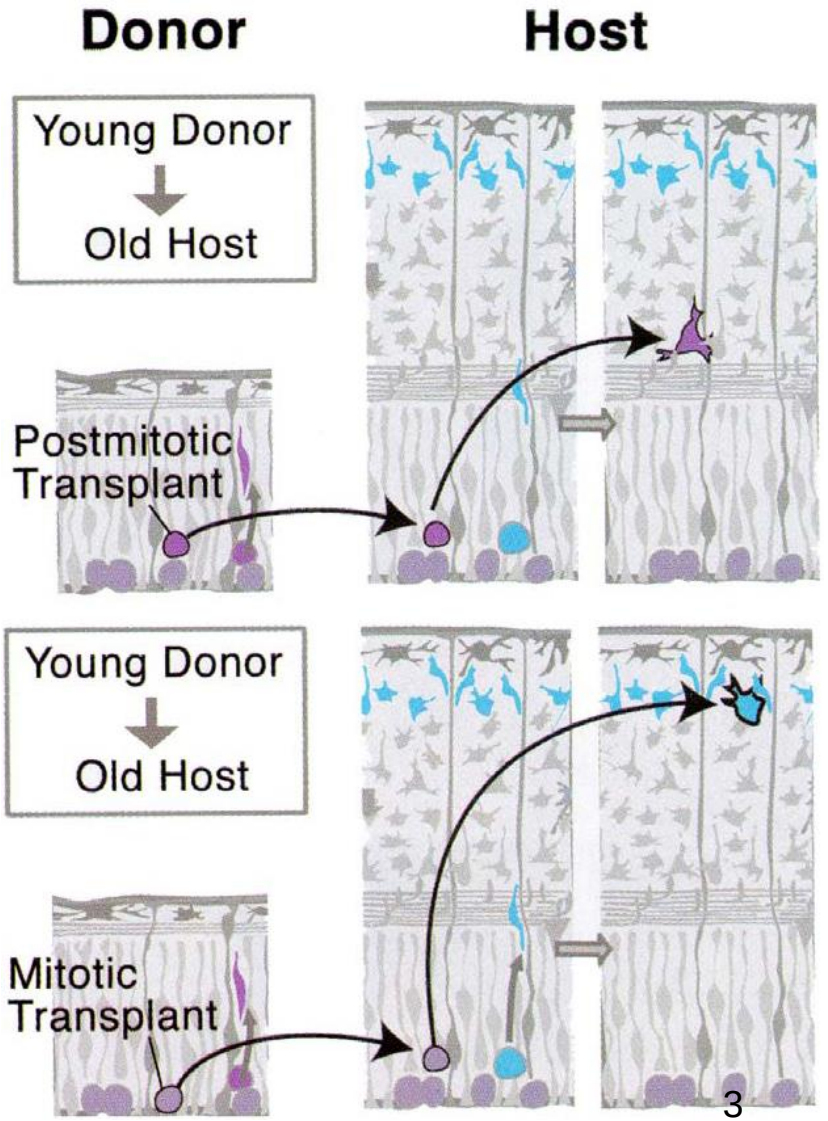
Course News

Friday, Oct 5 paper discussion
(do not come unless you have read the paper)

Monday, Oct 8 discussion report due in class

Environment's role in determining cell fate requires cell division.

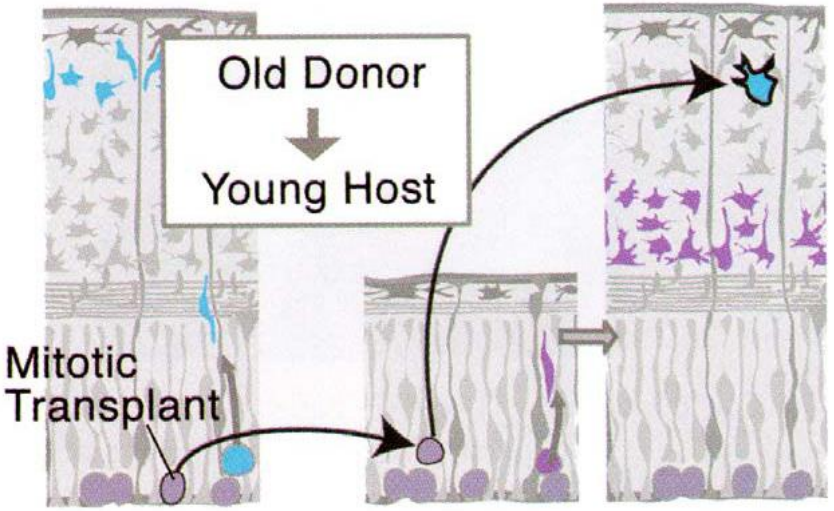
- Transplantation of E29 ventricular layer cells from ferret cortex to the ventricular layer of P1 cortex resulted in transplanted cells differentiating as layer 2-3 neurons or layer 6 neurons.
- Cells that did not divide in the host brain went to layer 6.
- Cells that divided in the host brain went to layer 2-3.



Changing progenitor cell competence is unidirectional.

- Transplantation of P1 ventricular layer cells to E29 ventricular layer resulted in transplanted cells differentiating as layer 2-3 neurons.

  i.e. The competence of the progenitor cells had irreversibly changed.



Molecular Basis of Determination

- All cells of an individual organism have the same genes.
  ... different cell characteristics result from expression of a particular set of genes.

- The set of functional proteins expressed by each cell type is determined by the specific complement of transcription factors expressed by the cell.

- Transcription factors promote or repress expression of specific genes.

- Each gene can have binding sites in its regulatory domain for many transcription factors.

- Different combinations of a limited number of transcription factors result in many different cell phenotypes.

Molecular Basis of Determination

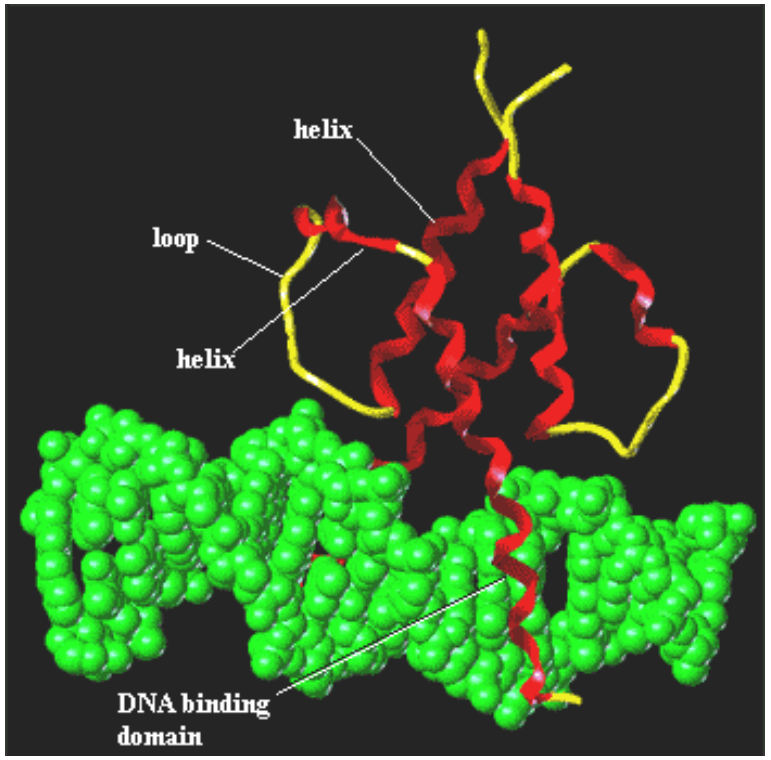
- Expression of a specific set of transcription factors specifies each cell fate.

Molecular Basis of Determination

- Expression of a transcription factor can define a whole system, a specific tissue or a single cell type:
  Sox2 = developing nervous system
  Pax6 = developing retina
  Nrl = developing rod photoreceptor neuron

Molecular Basis of Determination

- Beta helix-loop-helix (bHLH) transcription factors:
  - bHLH factors form their DNA recognition domain by dimerization.
  - bHLH factors bind a hexa-nucleotide sequence called the E-box, which is in the regulatory region of many genes involved in neuronal differentiation.
  - Functional dimmers are typically between a ubiquitously expressed class A bHLH factor (also called E-proteins) and a highly regulated class B bHLH factor.



Molecular Basis of Determination

- bHLH proneural transcription factors are linked to initiation of differentiation and neuronal fate:
  - Several class B bHLH factors are expressed in the developing nervous system, the proneural genes. These include Ash1 (Ascl1), Ath1 (Atoh1), Ath5 (Atoh7), Nrg 1, 2 & 3.

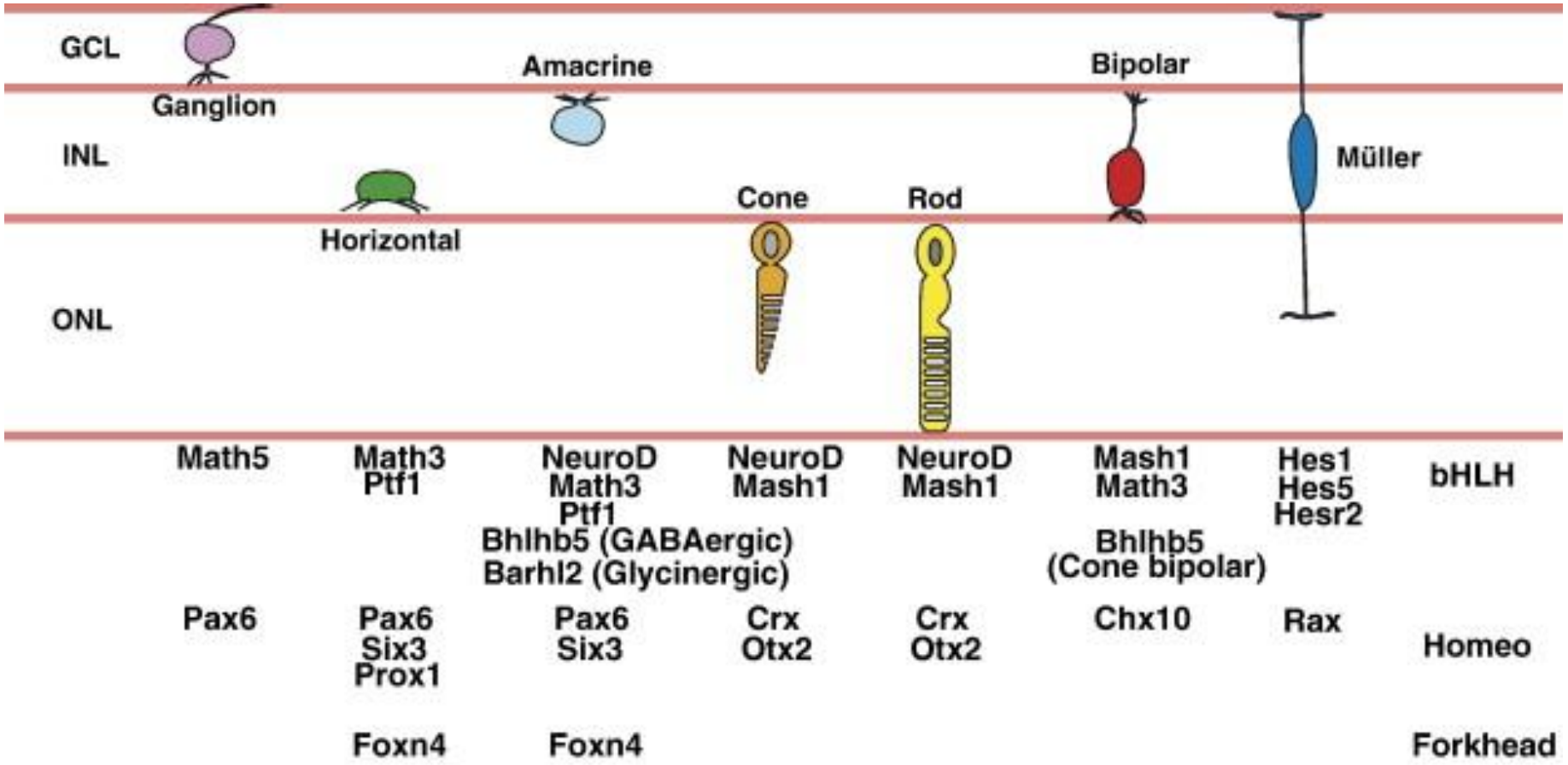
    [prefix M=mouse, X=xenopus, C=chick, etc.]
  - Misexpression of certain proneural genes drive progenitor cells to differentiate.
  - Knockout of a single proneural gene can result in failure of specific neuronal types to develop.

Molecular Basis of Determination

- Other families of transcription factors also are involved in cell fate determination including homeodomain factors and forkhead factors.

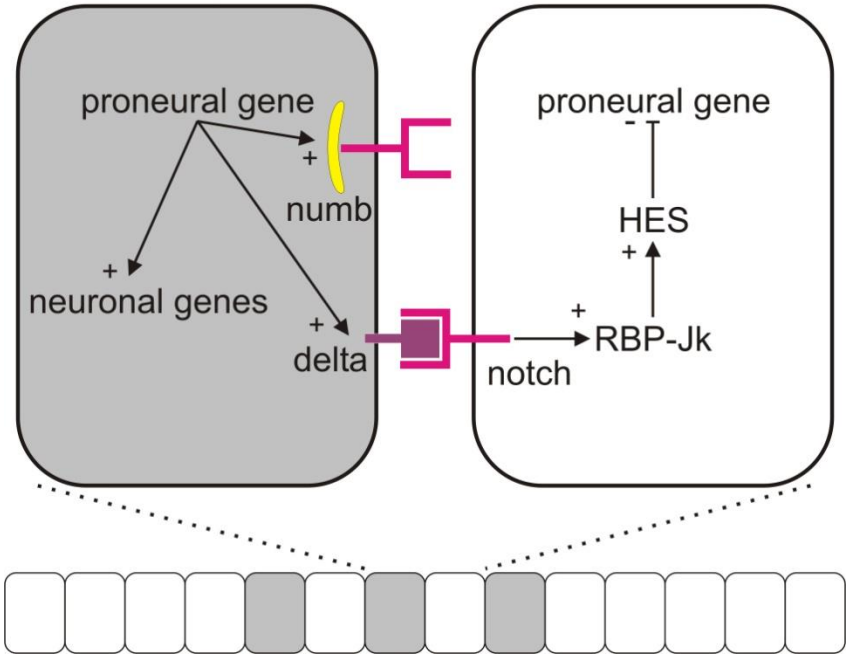
Molecular Basis of Determination

- Seven retinal cell types are each determined by expression of a specific complement of transcription factors.



Molecular Basis of Determination

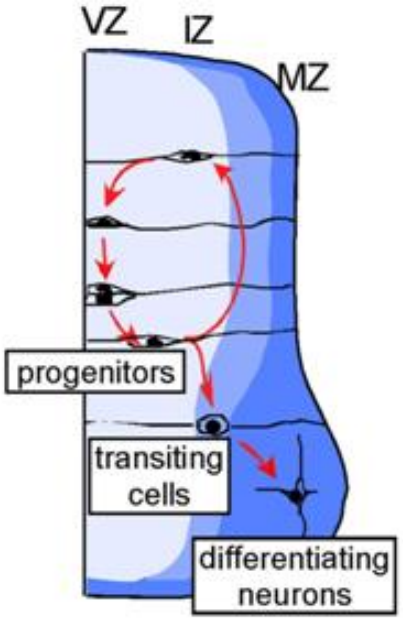
- Notch and proneural factors:
  - Expression of bHLH proneural factors promotes expression of the Notch ligand, Delta; Delta activates Notch on neighboring progenitor cells.
  - Notch activation represses expression of bHLH proneural factors and prevents differentiation.
  - Notch activation can lead to demethylation of certain genes in progenitor cells.
  - Thus, Notch activation can change progenitor cell competence.

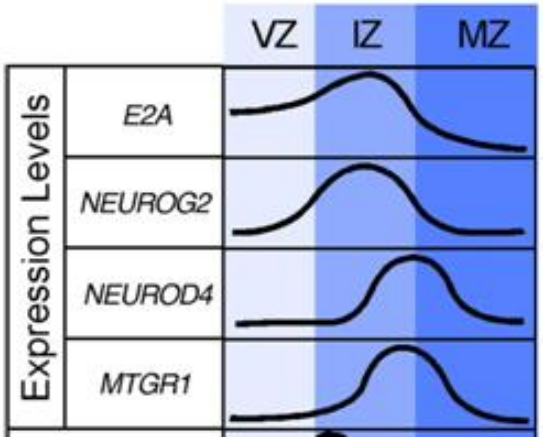


Molecular Basis of Determination

- Cells change the transcription factors expressed as they differentiate.

  i.e. Different transcription factors direct different stages of differentiation.

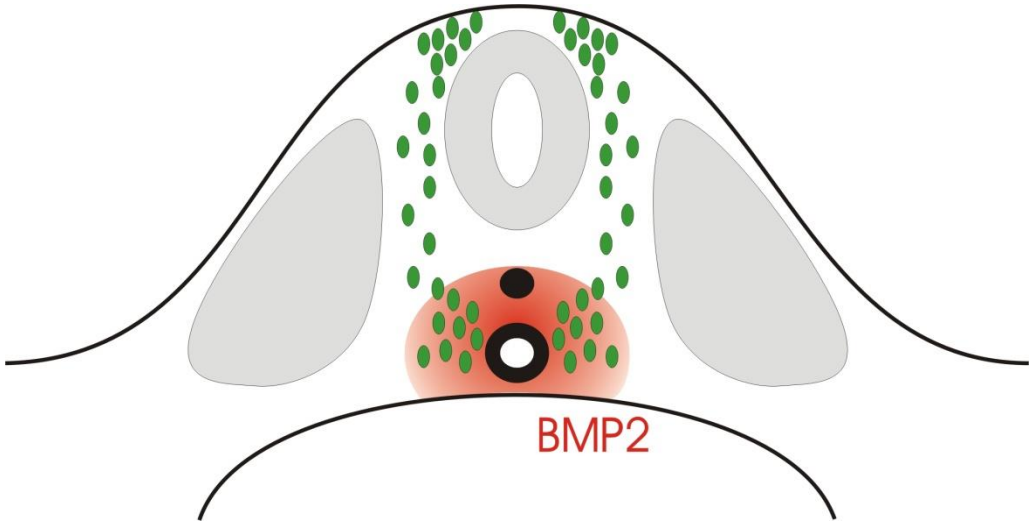




Inducing factors promote expression of specific transcription factors.

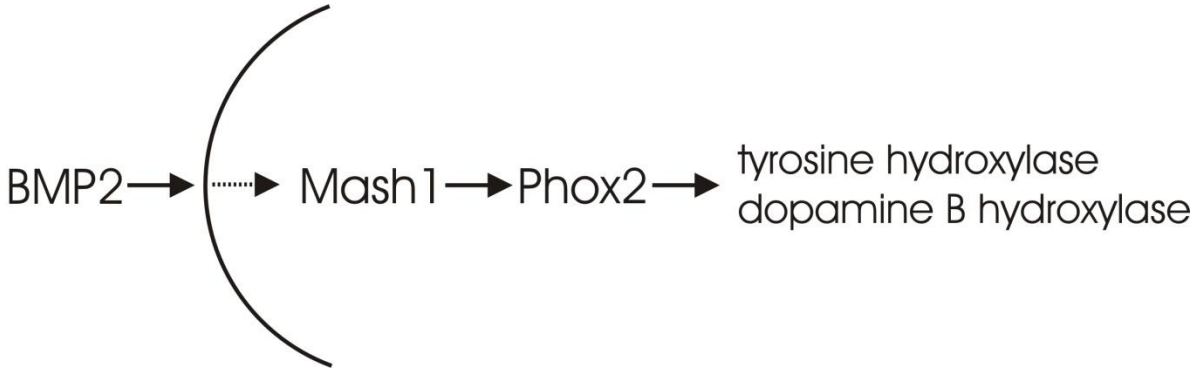
e.g. sympathetic ganglion neurons (neural crest)

- Crest cells destined to become autonomic ganglion neurons express Ash1 (a bHLH transcription factor).
- No sympathetic ganglia neurons develop without Ash1.
- Ash1 expression is induced by BMP2 released from the aorta.



Specific transcription factors promote expression of proteins that define cell fate.

- Ash1 promotes expression of Phox2 (a homeodomain transcription factor).
- Early sympathetic neurons express tyrosine hydroxylase and dopamine B hydroxylase. Sympathetic neurons require expression of Phox2 to express these enzymes.
- Phox2 binds to the promoter region of the genes for these enzymes.
- Notochord also is required for expression of Phox2 in the developing sympathetic ganglion, possibly for Shh.

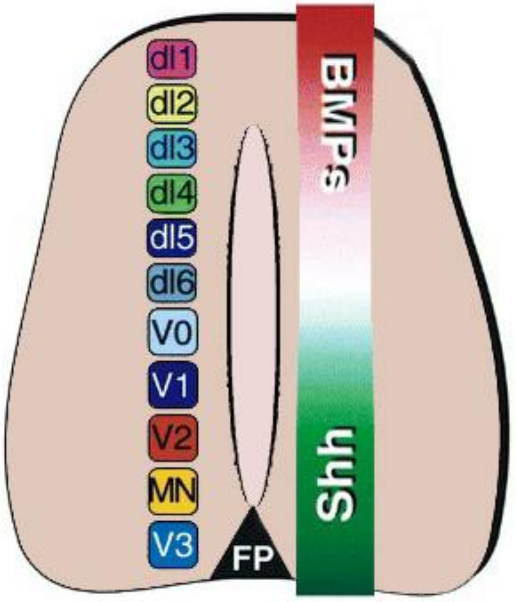


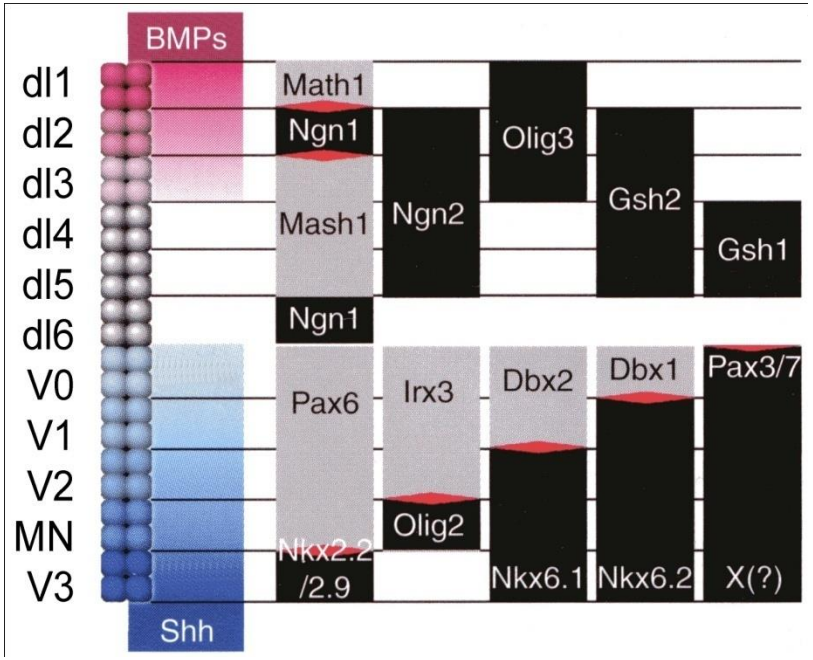
Inducing factors promote expression of specific transcription factors.

e.g. spinal cord

- The relative levels of Shh and BMPs determine the transcription factors expressed in each dorsal-ventral domain of the developing spinal cord.

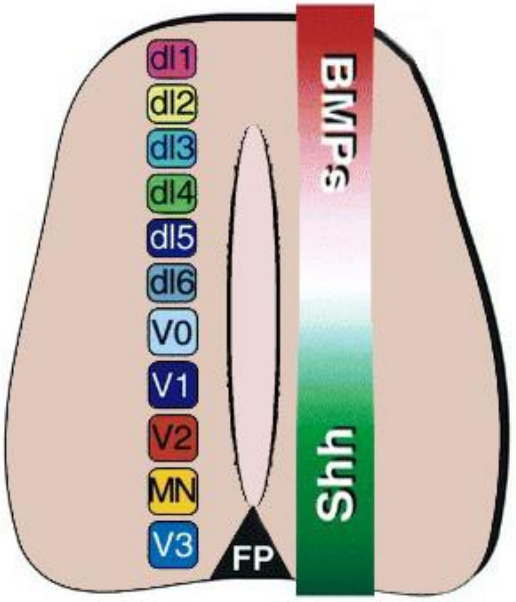
- The combination of factors expressed in each domain determines the cell types that develop there.

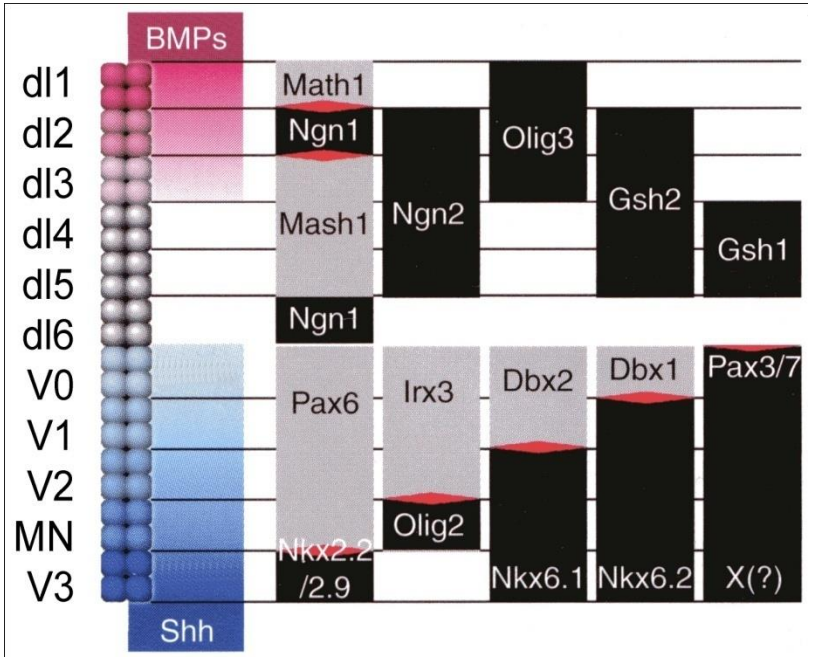




Inducing factors promote expression of specific transcription factors.

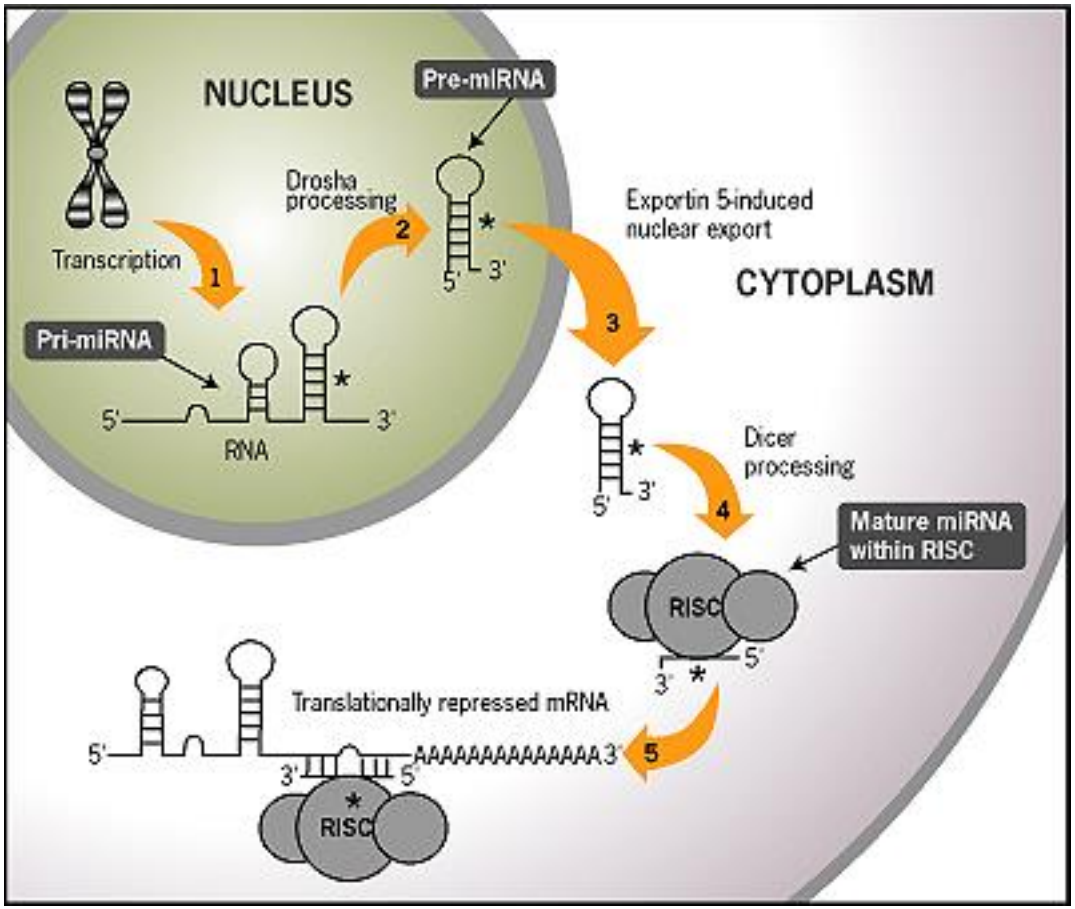
- Ath1(Math1) is required for development of dI1 neurons.
- When Ath1 was ectopically expressed in dorsal cord, dI1 neurons were generated at the expense of other neuron types.
- When Ath1 was ectopically expressed in ventral cord, dI1 neurons were not generated.
  i.e. other factors are also required, which are not in ventral cord.





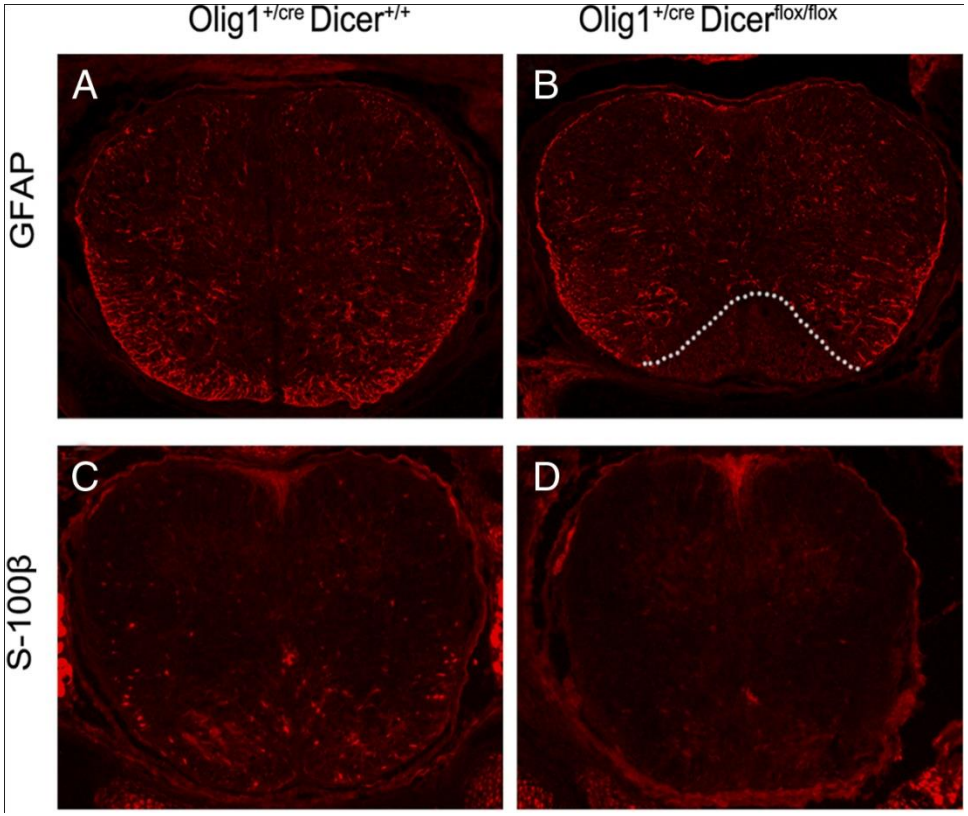
MicroRNAs Involved in Cell Fate

- MiRNAs are non-coding RNAs, 18-25 nucleotides long. MiRNAs are cleaved from longer RNAs in a multi-step process requiring the enzyme, Dicer. MiRNAs hybridize with mRNA, thus blocking translation and/or targeting them for degredation.
- Most of the 462 miRNAs identified (in humans) are expressed in the brain, and most of these are developmentally regulated.



MicroRNAs Involved in Cell Fate

- A conditional kockout of dicer in the ventral spinal cord of developing mice reduced astrocycte genesis and virtually eliminated oligodendrocyte genesis.



(GFAP = astrocyte, S100 = oligodendrocyte)
Zheng K et al., 2010

MicroRNAs Involved in Cell Fate

Factors that maintain cells as neural progenitors:

- Repressor Element Silencing Factor (REST)
  -also called-
  Neuron-Restrictive Silencer Factor (NRSF)

- RNA polymerase II C-Terminal Domain Small Phosphatase (CTDSP)

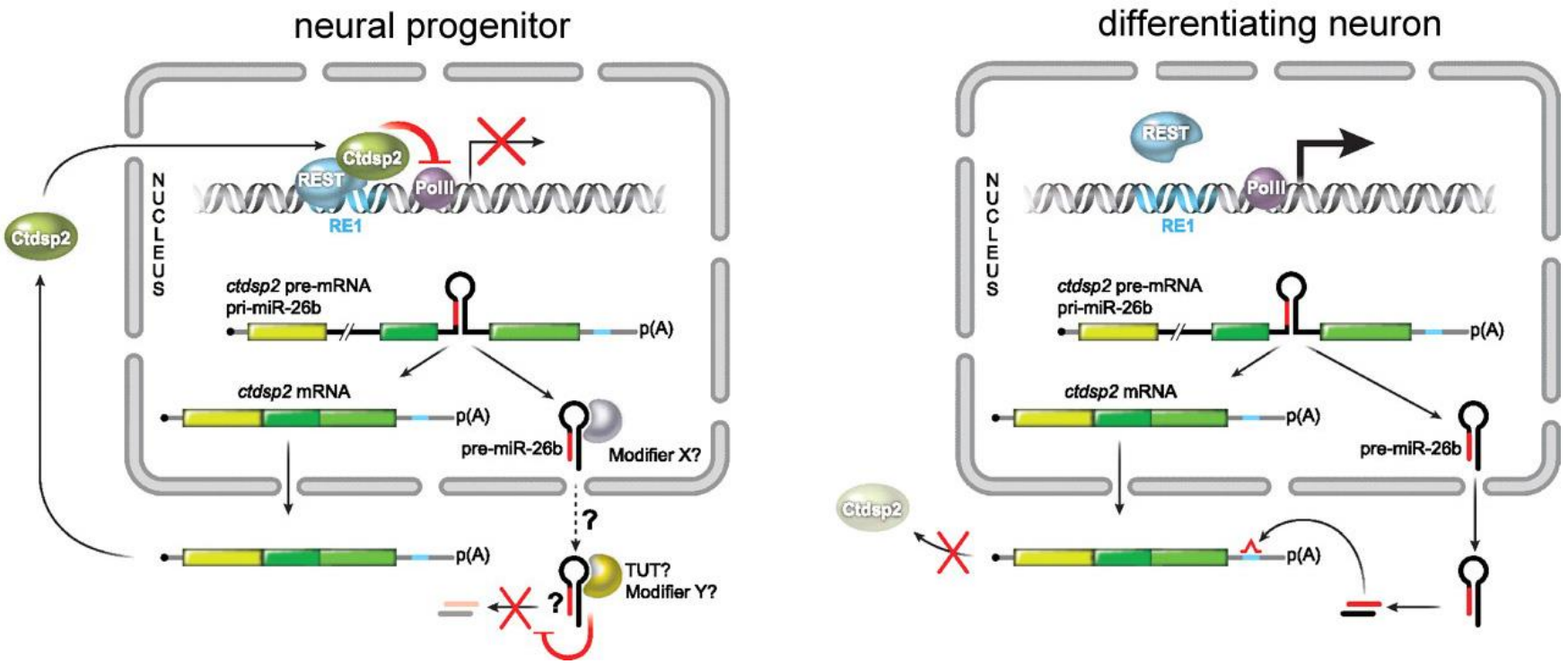
RNA polymerase II activity is needed for expression of neuronal genes.

REST/CTDSP block RNA polymerase II.

miR-26b blocks translation of CTDSP.

miR-26b is in an intron of the CTDSP mRNA.

MicroRNAs Involved in Cell Fate



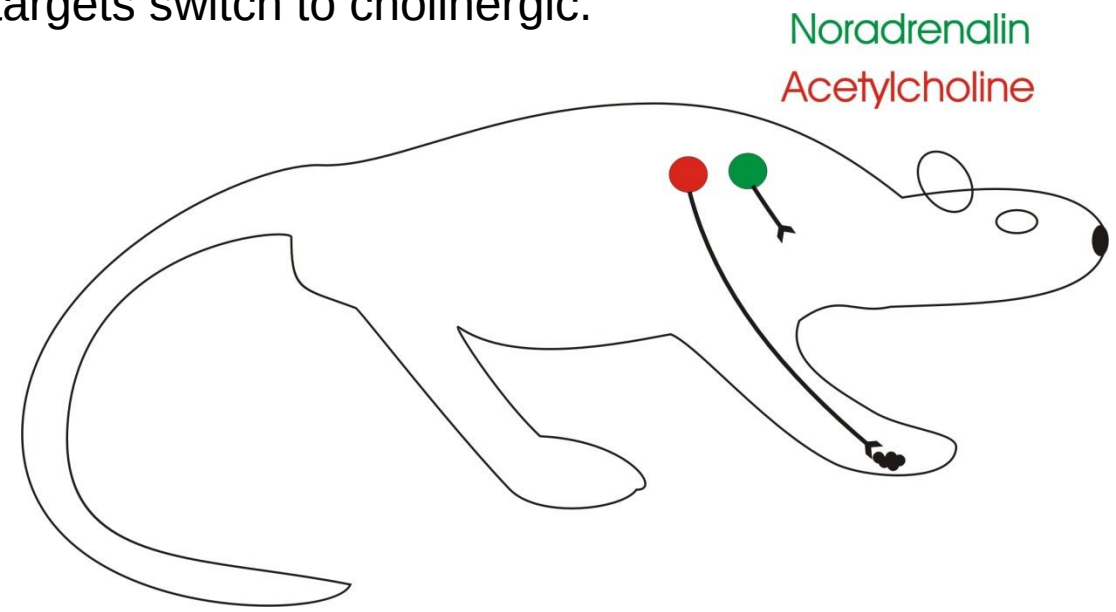
Dill H et al. (2012) *Genes Dev.* 26:25
Han J et al. (2012) review

Cell-cell interactions can influence the final phenotype of a cell late in development.

e.g. sympathetic neuron transmitter

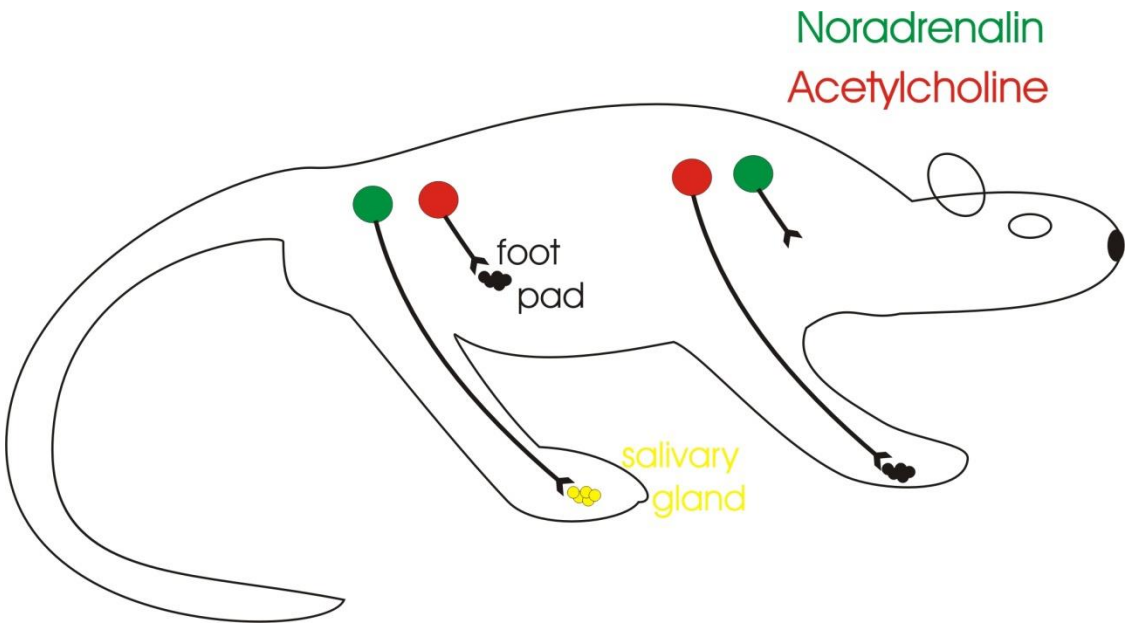
- Initially in development, all sympathetic neurons are noradrenergic.

- After their axons innervate target tissues, those neurons projecting to foot pad sweat glands and some other targets switch to cholinergic.



Cell-cell interactions can influence the final phenotype of a cell late in development.

- Transplants suggest that the target tissue specifies the transmitter phenotype.



Cell-cell interactions can influence the final phenotype of a cell late in development.

- Tabby mice w/o foot pad glands lack cholinergic sympathetic neurons, i.e. activity is in gland & not surrounding tissue.

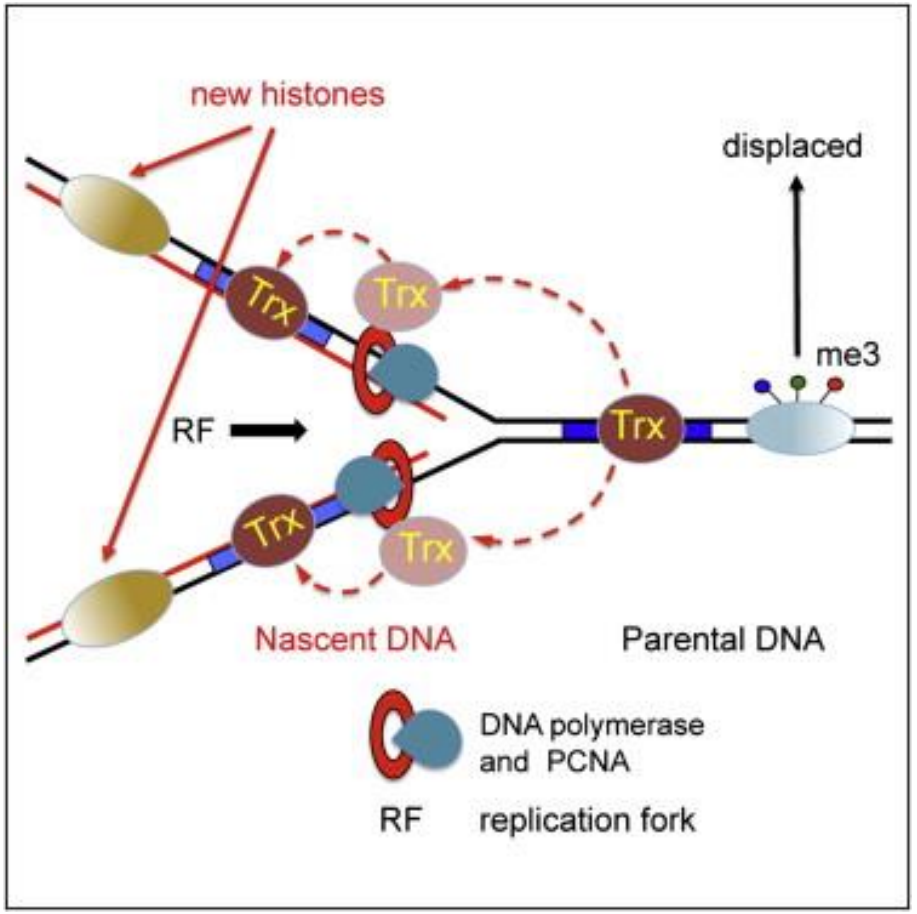
- In tissue culture, ciliary neurotrophic factor (CNTF) induced sympathetic neurons to switch to the cholinergic phenotype.

- Antibodies to CNTF remove cholinergic inducing activity from footpad extract.

Cell-cell interactions can influence the final phenotype of a cell late in development.

- However, CNTF does not appear to be expressed in the developing foot pad glands.

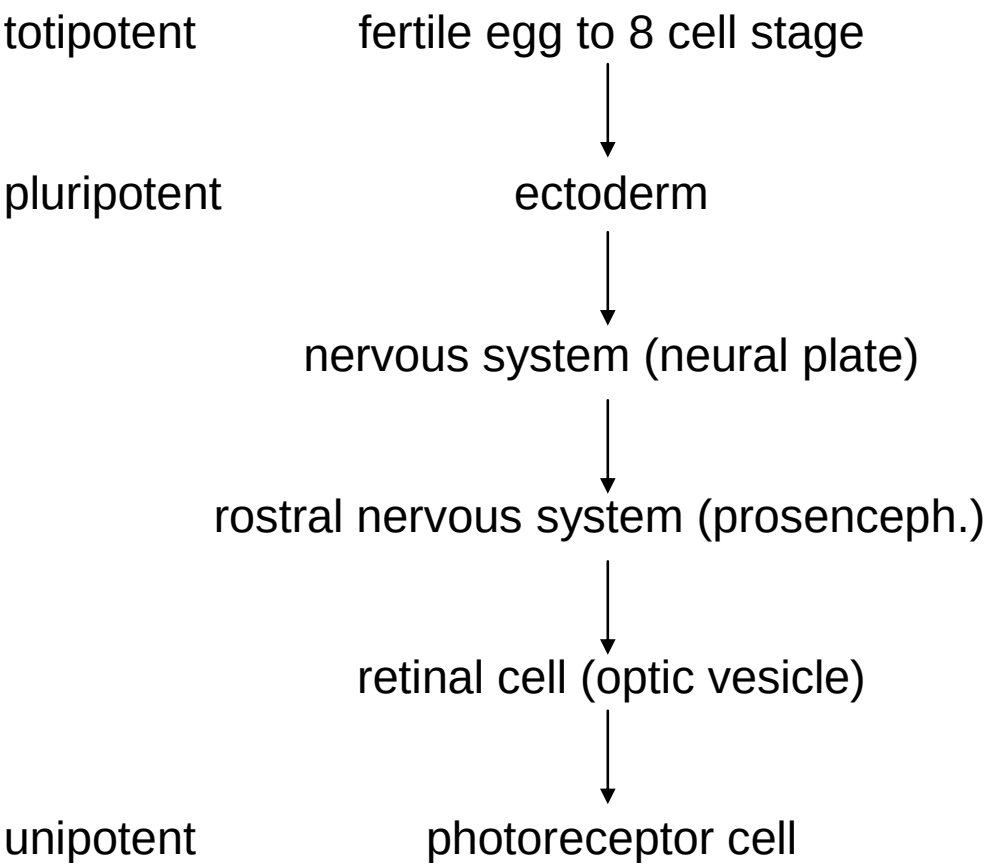
Histone Modification through Cell Division



Petruk S et al., 2012, Cell 150:922

Concept …

Determination of cell fate is due to the sequential restriction of possible fates:



Concept …

Sequential steps in the restriction of possible fates are controlled by different mechanisms.

Early steps: Differences among cells through the blastula stage are due to asymmetric distribution of maternal mRNAs during cell division.

Mid steps: Large scale patterning of tissues is due to secretion of inducing factors that act over large distances. Generally factors released from one population of cells acts on another population of cells. These factors are often in gradients and the relative concentration of a factor determines its effect. Generally it is the sum action of multiple factors that defines the nature of a cell.

Final steps: The possible fates of differentiated cells in a tissue are determined by the previous patterning events (i.e. early and mid steps). Local cell-cell interactions specify the fate of individual cells. Local cell-cell interactions can be a combination of secreted factors and cell contact mediated signaling systems.