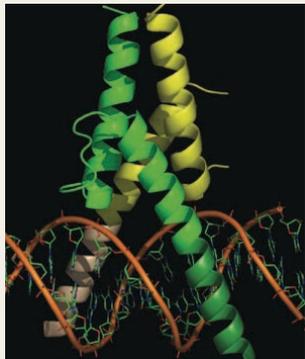
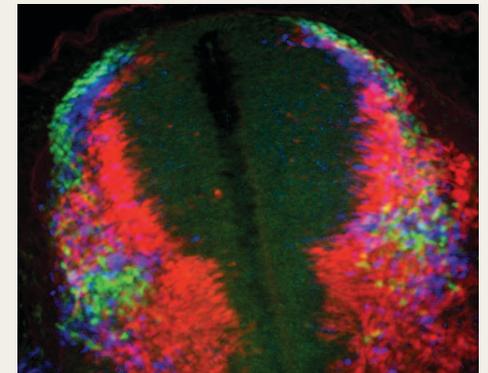
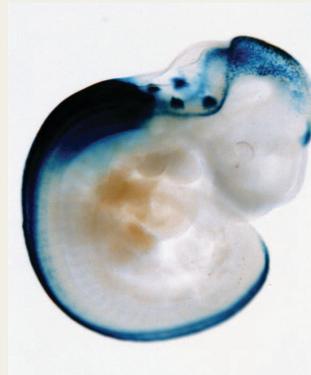
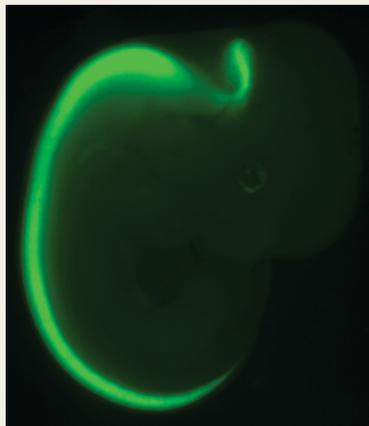


Transcriptional Control of Neural Diversity



Jane E. Johnson, PhD
UT Southwestern Medical Center
Department of Neuroscience

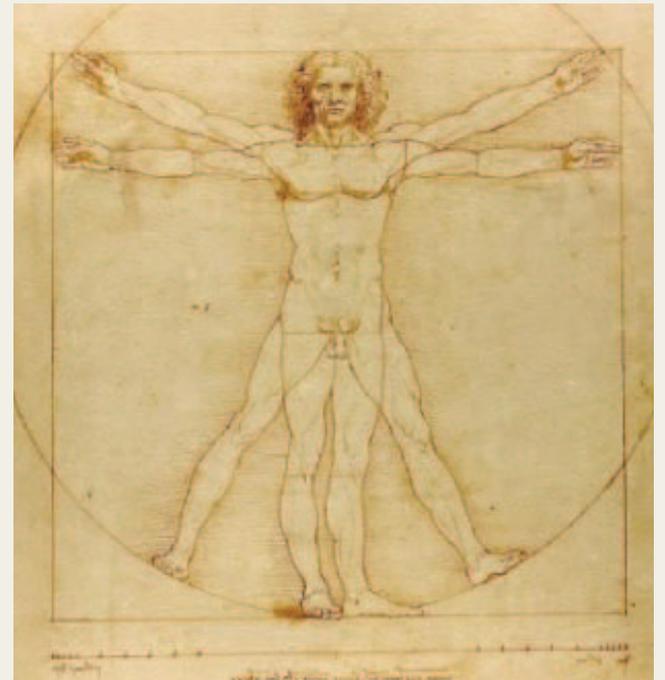




??Eyles??|??|??|??|??



?i ?Evd?

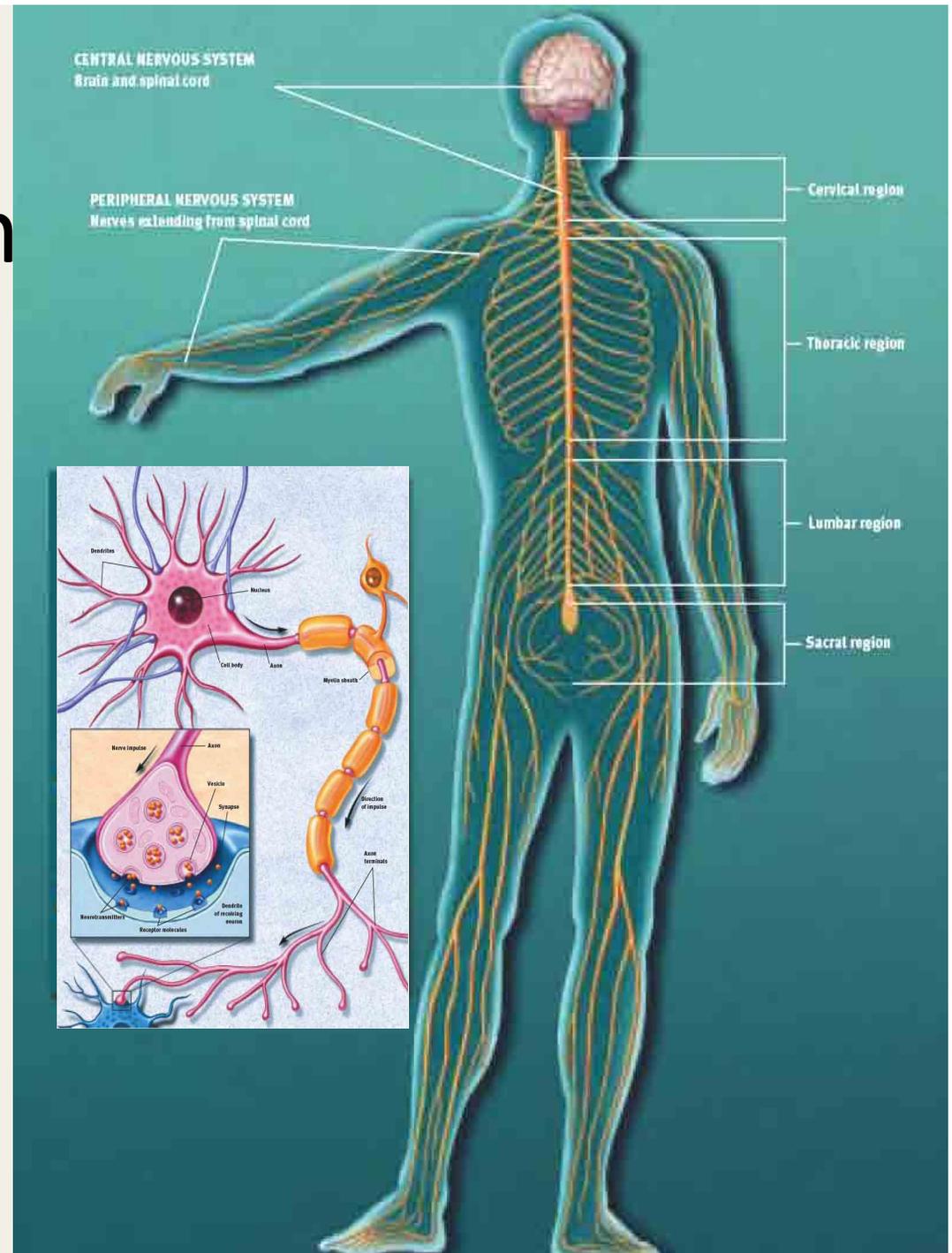


??Alb?

Nervous System

- Brain
- Spinal cord
- Sensory systems

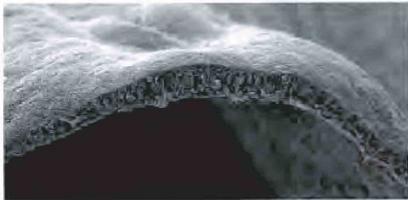
- Neurons
- Oligodendrocytes
- Astrocytes



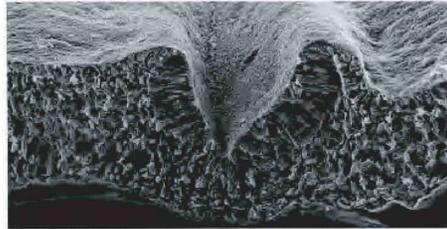
Nervous System Development

A. Developmental Stages

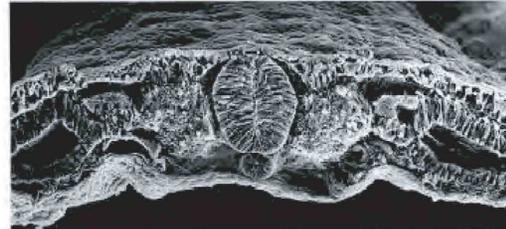
Neural Plate



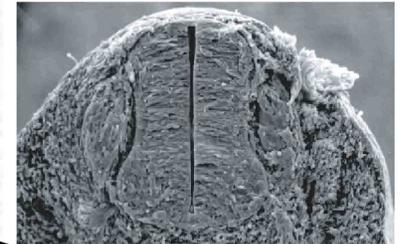
Neural Fold



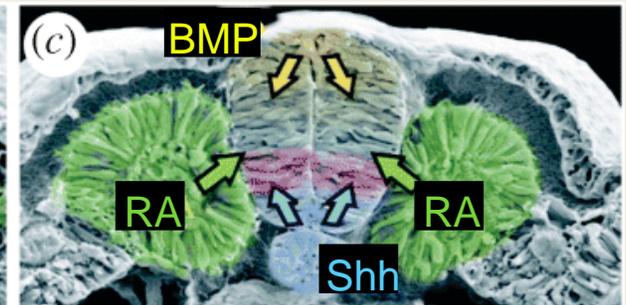
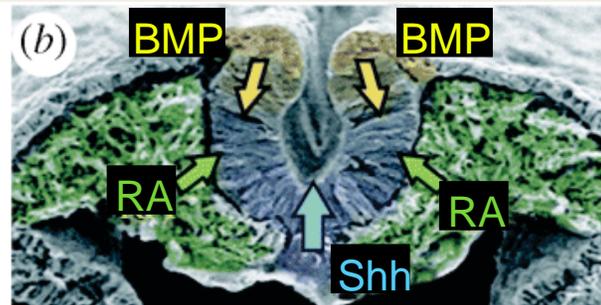
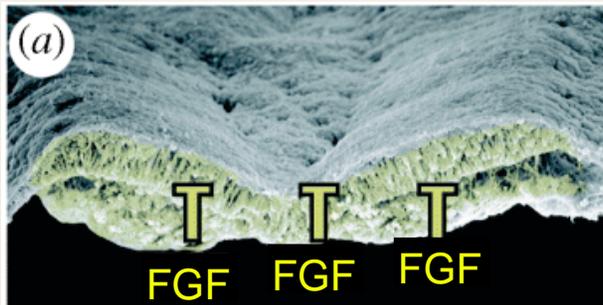
Neural Tube



Spinal Cord

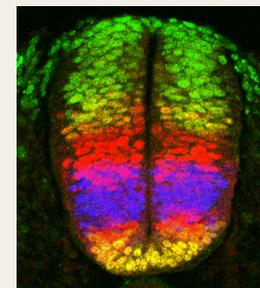


B. Signaling factors pattern the neural tube



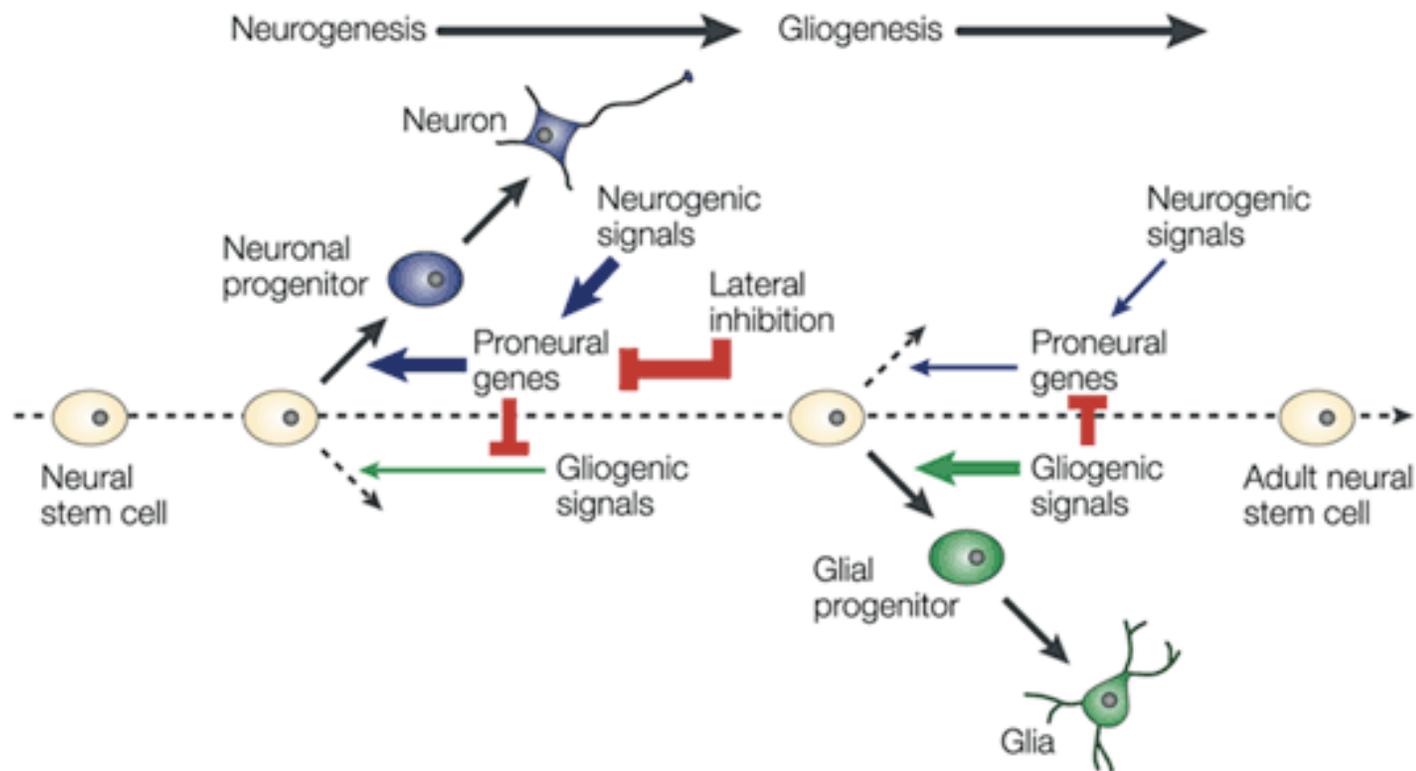
© Eric D. Snider, 2002. All rights reserved.

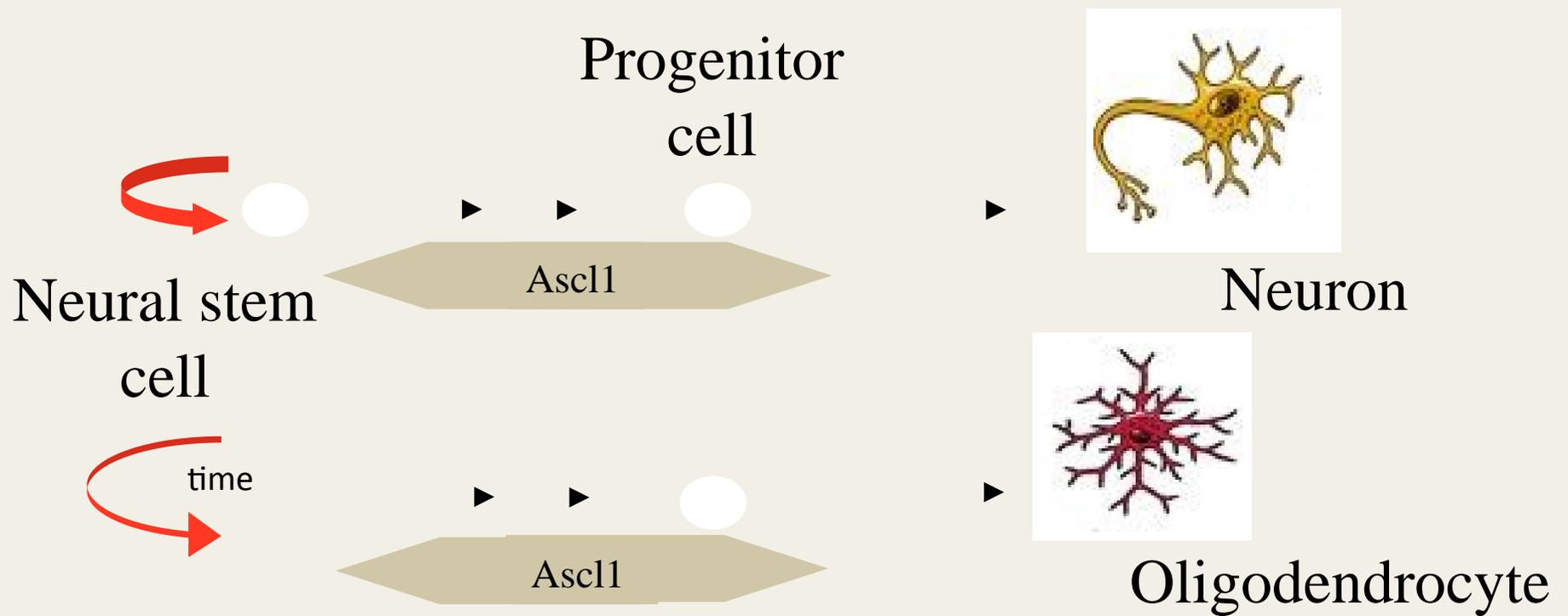
C. Transcription factor expression is patterned by signaling factors (particularly homeodomain and bHLH classes).



© Eric D. Snider, 2002. All rights reserved.

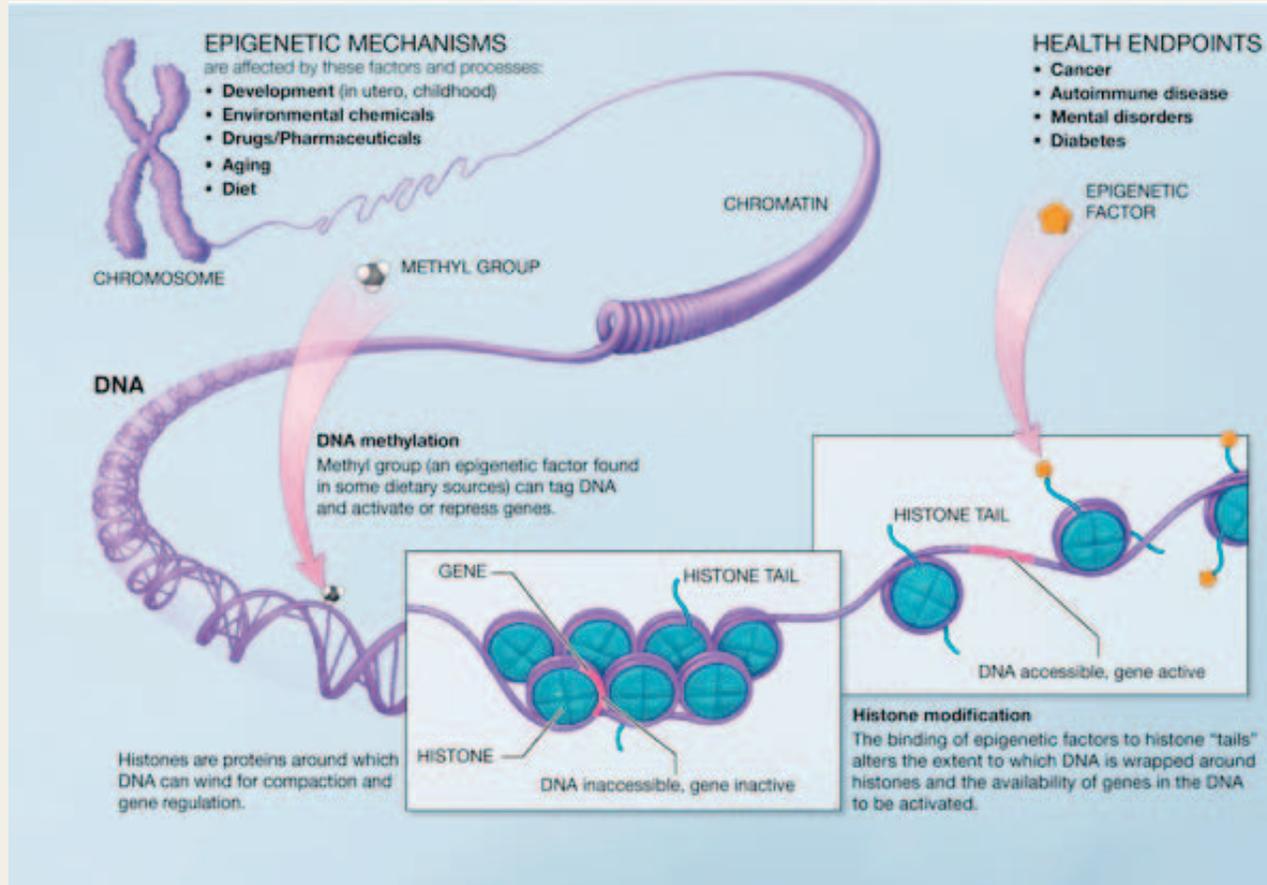
Overview of the generation of different neural cell types during development





Ascl1 is expressed in and required for subsets of neuronal and oligodendrocyte progenitors

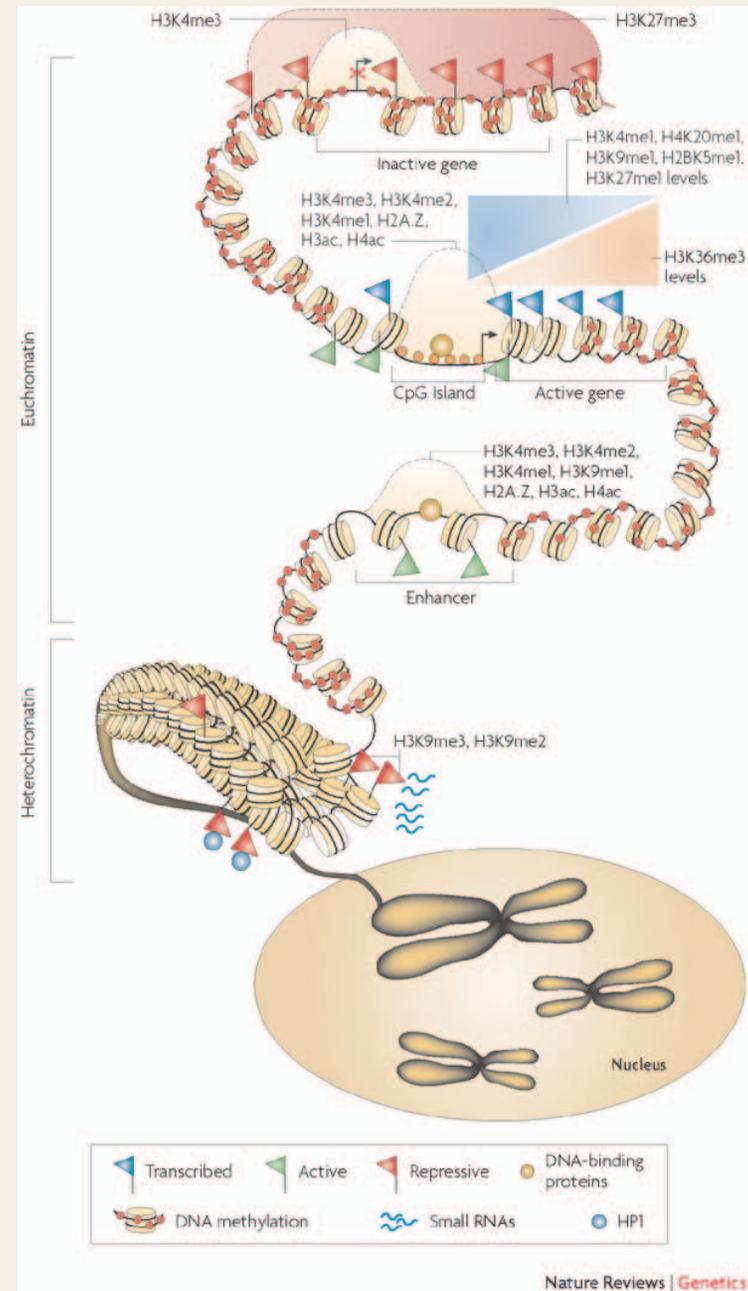
Transcription Factor Machinery and Epigenetics



In biology, and specifically genetics, **epigenetics** is the study of inherited changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence, hence the name *epi-* (Greek: $\overline{\square\square\square}$ over, above) -genetics. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism;^[1] instead, non-genetic factors cause the organism's genes to behave (or "express themselves") differently. (Wikipedia: epigenetics)

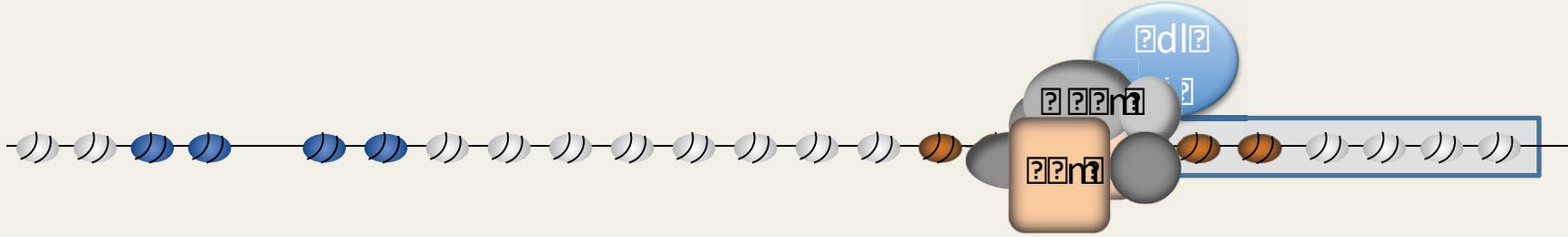
Transcription factor accessibility

- $\text{H3K4me3, H3K4me2, H3K4me1, H2A.Z, H3ac, H4ac}$
- $\text{H3K9me3, H3K9me2, H3K27me3}$
- H3K36me3

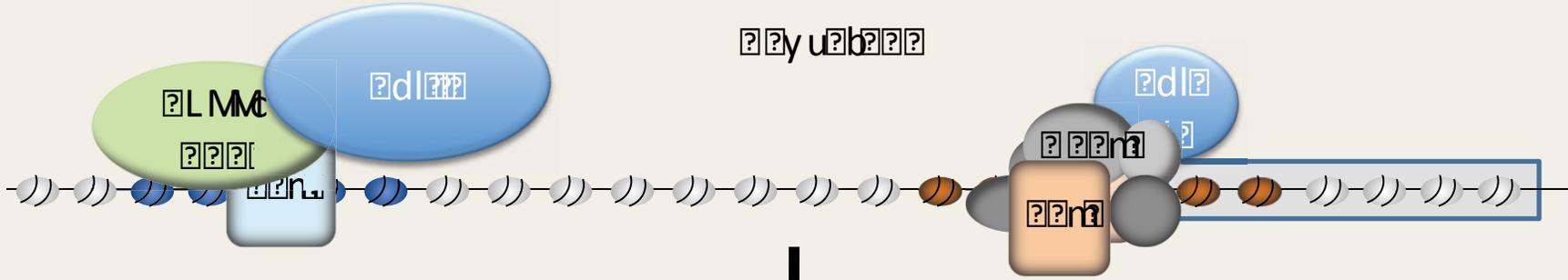


Any z p p F dz n 3 p p r e F 0
 2 2 b A H 2 2 u e t n 2 z y n D a
 f Z D 6 D f 3 1 2 2 F 3 W M H 2

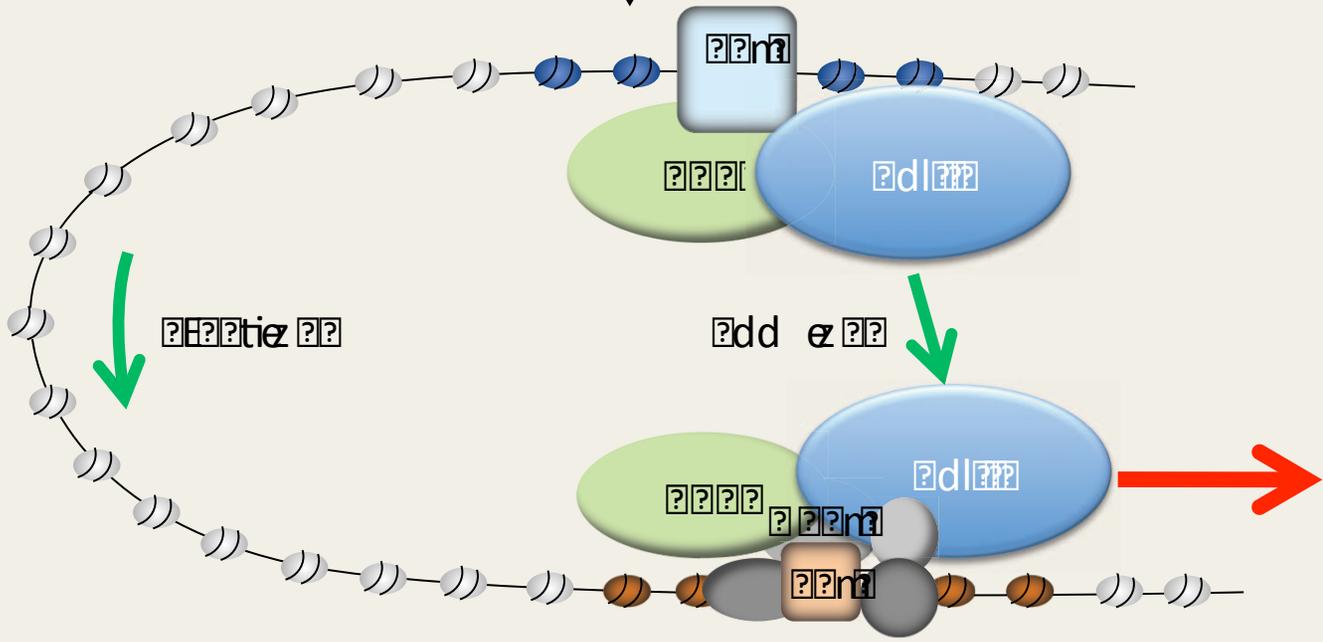
z F z z E O z z z z b z m E e y dz z



z F z E O z z b E m E e y d z



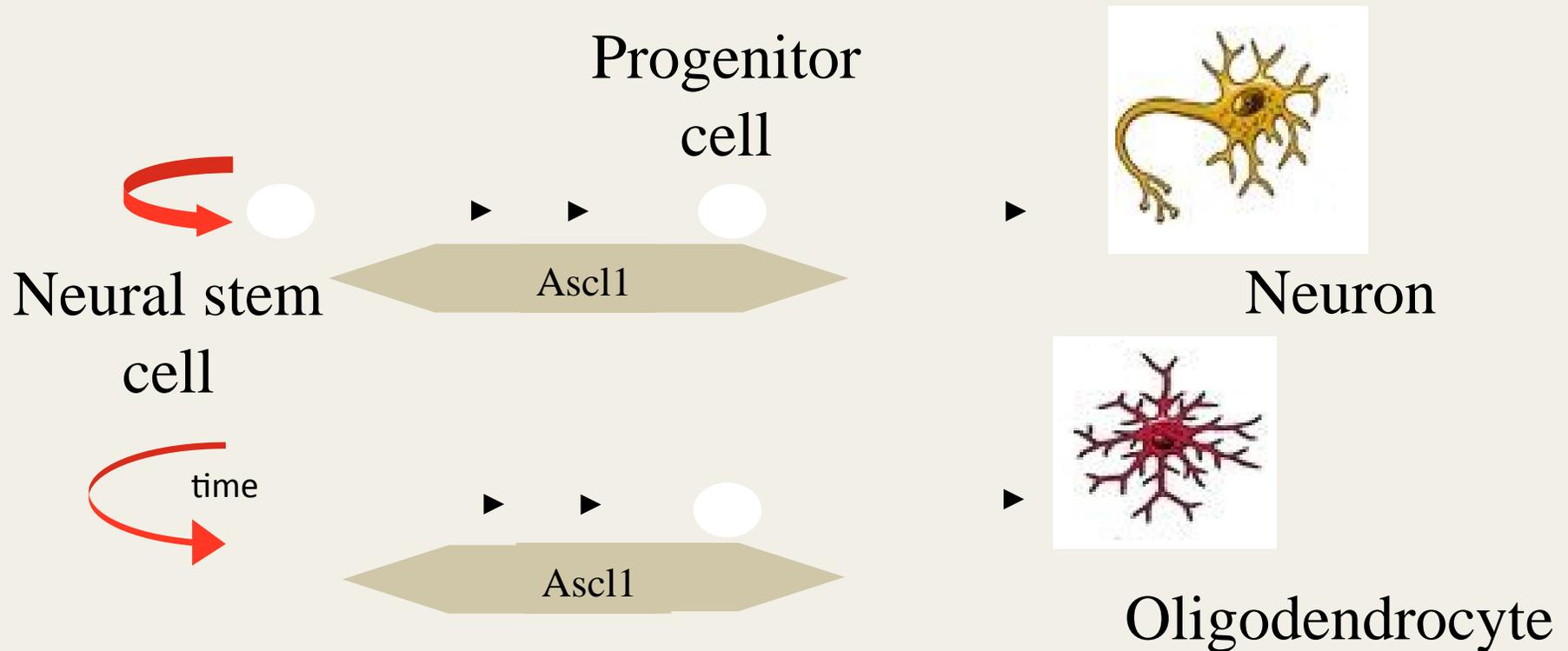
y u b



E z ?

E z ?

Back to the biological question of transcription control of neural development



Ascl1 is expressed in and required for subsets of neuronal and oligodendrocyte progenitors

Role of *Nr1f1* in the development of the cerebellum

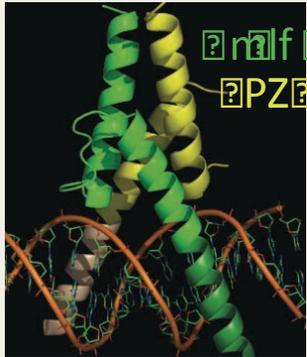


Figure 1: Structure of Nr1f1 protein.

→

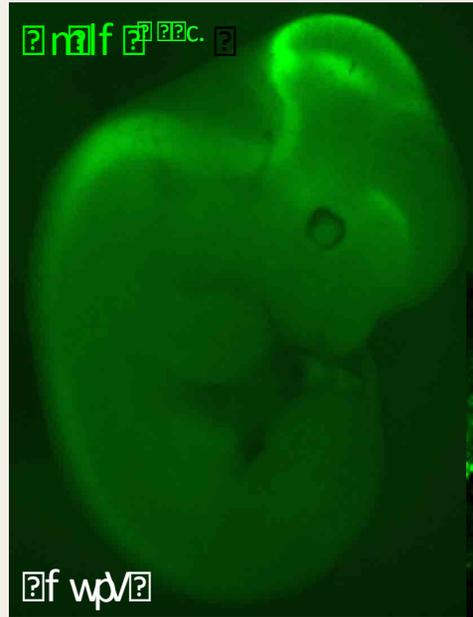


Figure 2: Whole-mount fluorescence image of a wild-type cerebellum.

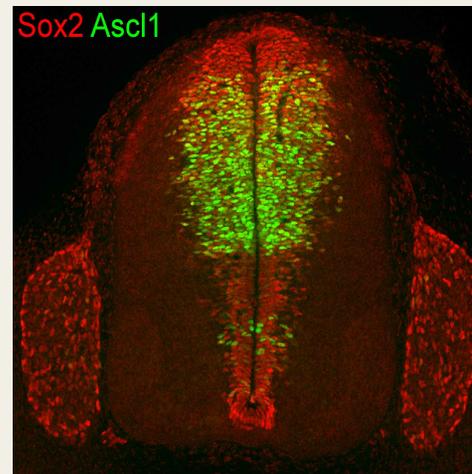


Figure 3: High-magnification fluorescence image of the SGZ in a wild-type cerebellum.

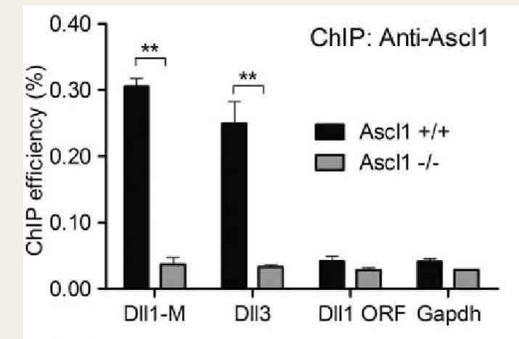
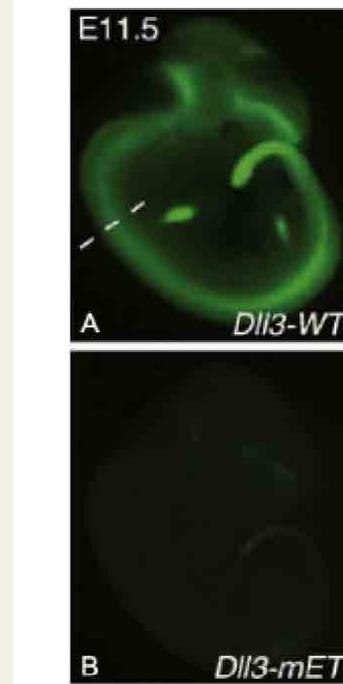
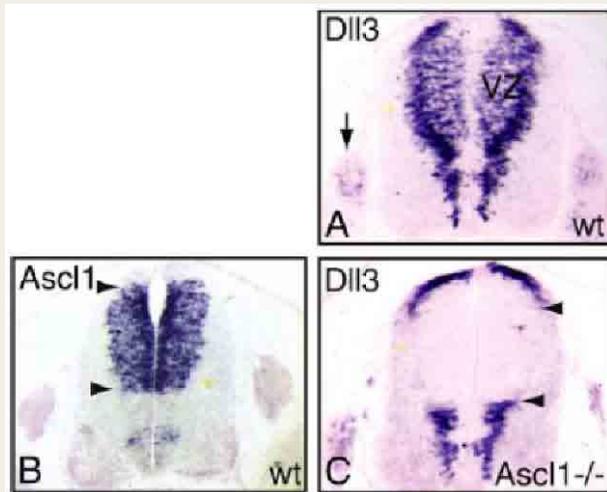
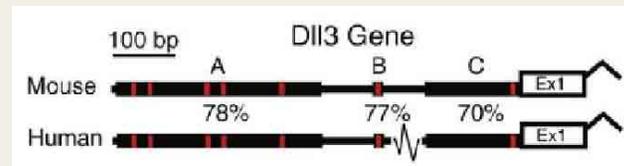
• *Nr1f1* is expressed in the granule cell layer of the cerebellum during development.

• *Nr1f1* is essential for the proliferation and differentiation of granule cell precursors in the SGZ.

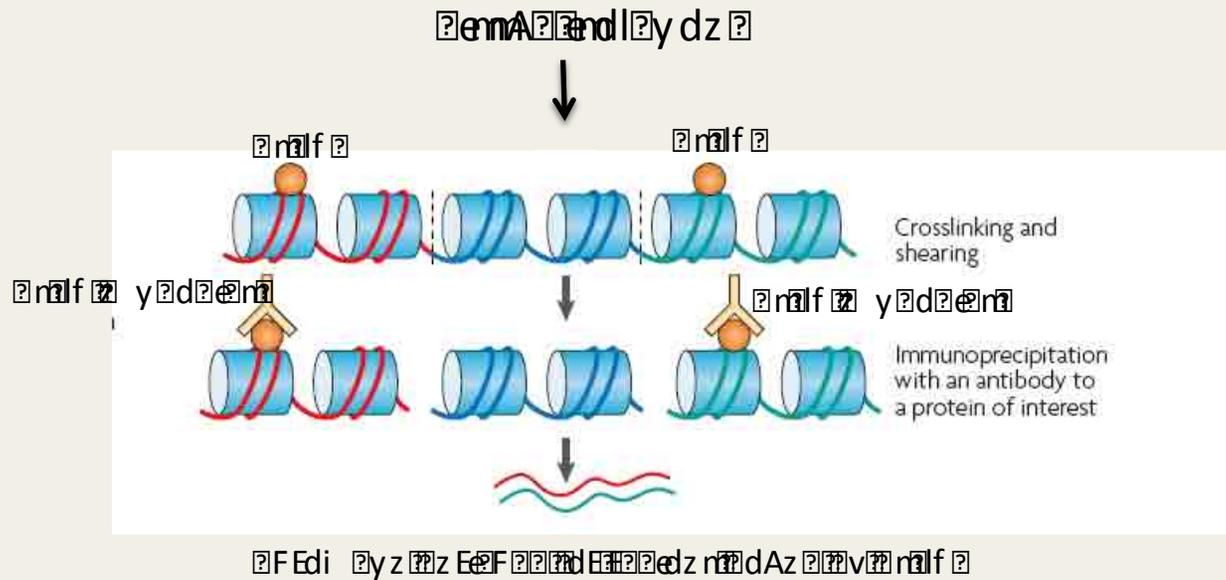
• *Nr1f1* is required for the proper development of the cerebellum.



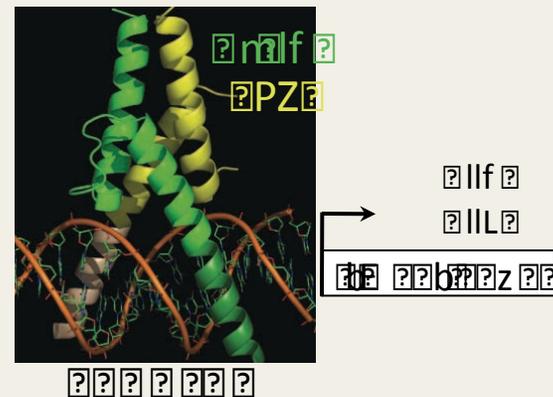
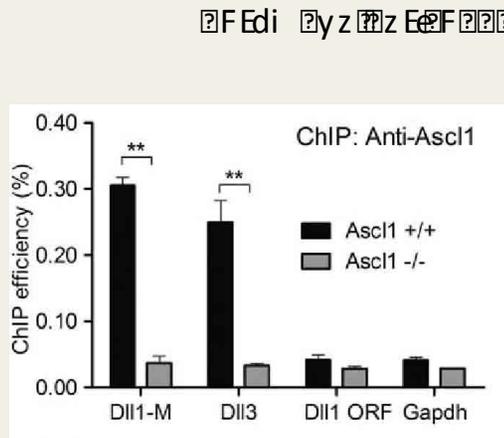
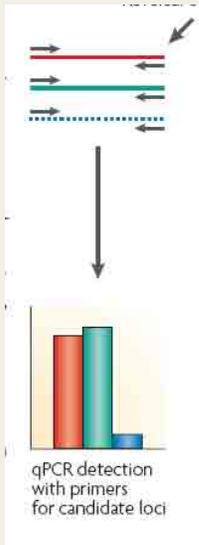
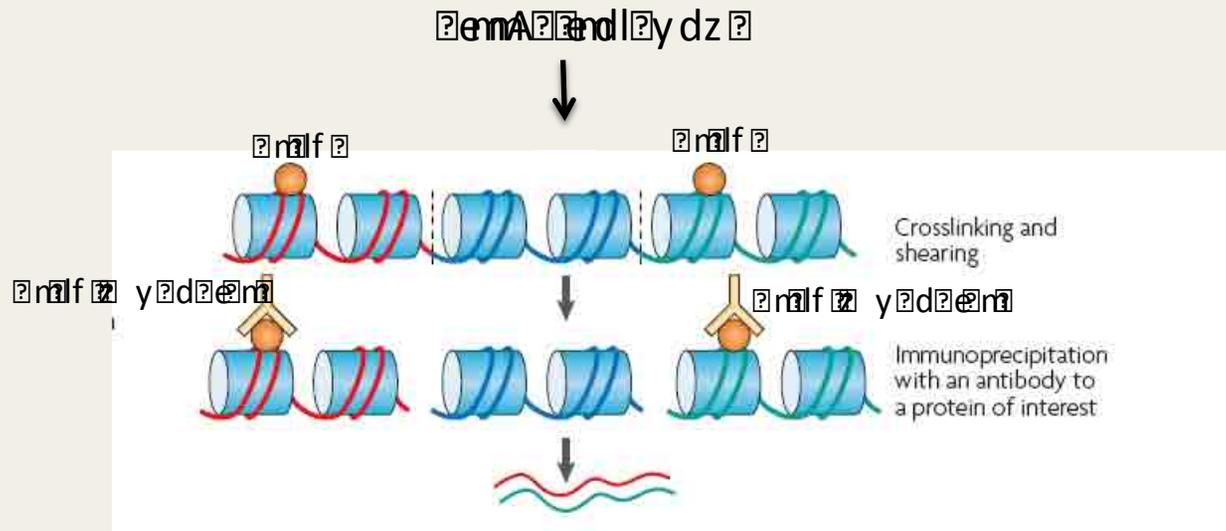
Ascl1 Regulates the Expression of Notch Ligand Delta-like 3 (DII3) through a proximal promoter



Chromatin Immunoprecipitation (ChIP)



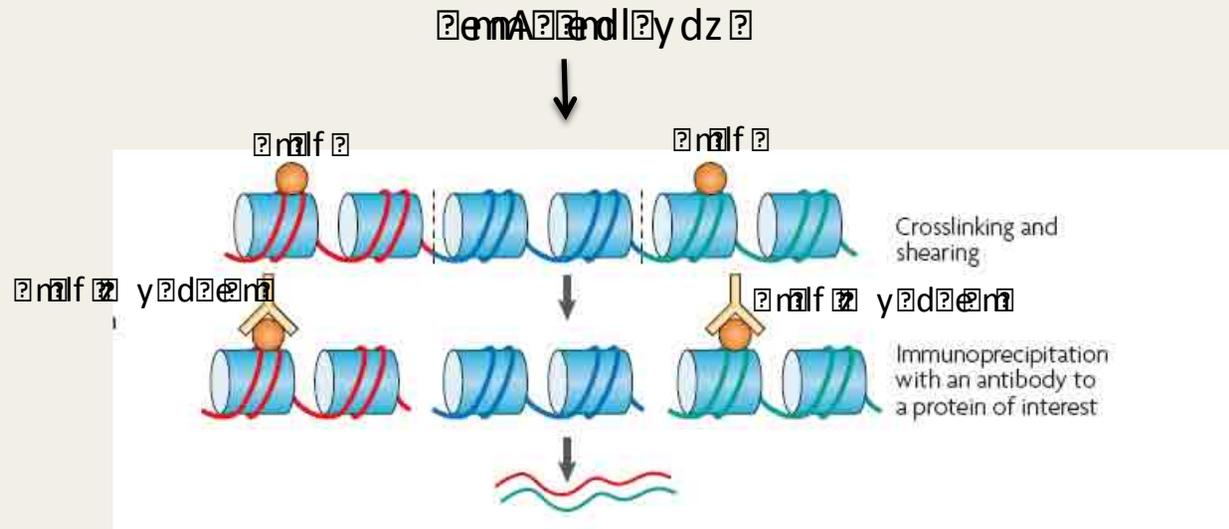
Chromatin Immunoprecipitation (ChIP)



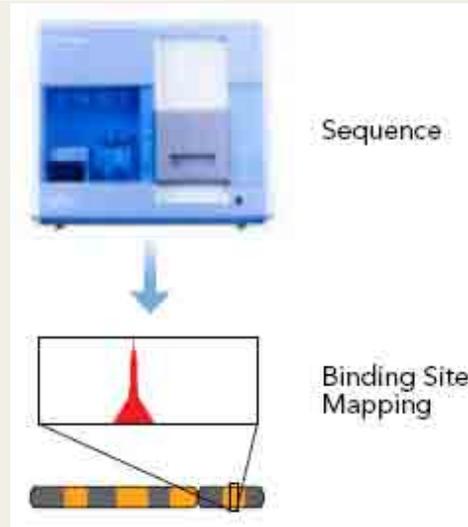
How to get a genomewide understanding of *Ascl1* targets?

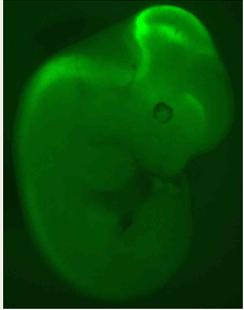
Chromatin Immunoprecipitation (ChIP)
followed by sequencing (ChIP-seq)

Genome-wide Analysis of Transcription Factor DNA Binding Sites

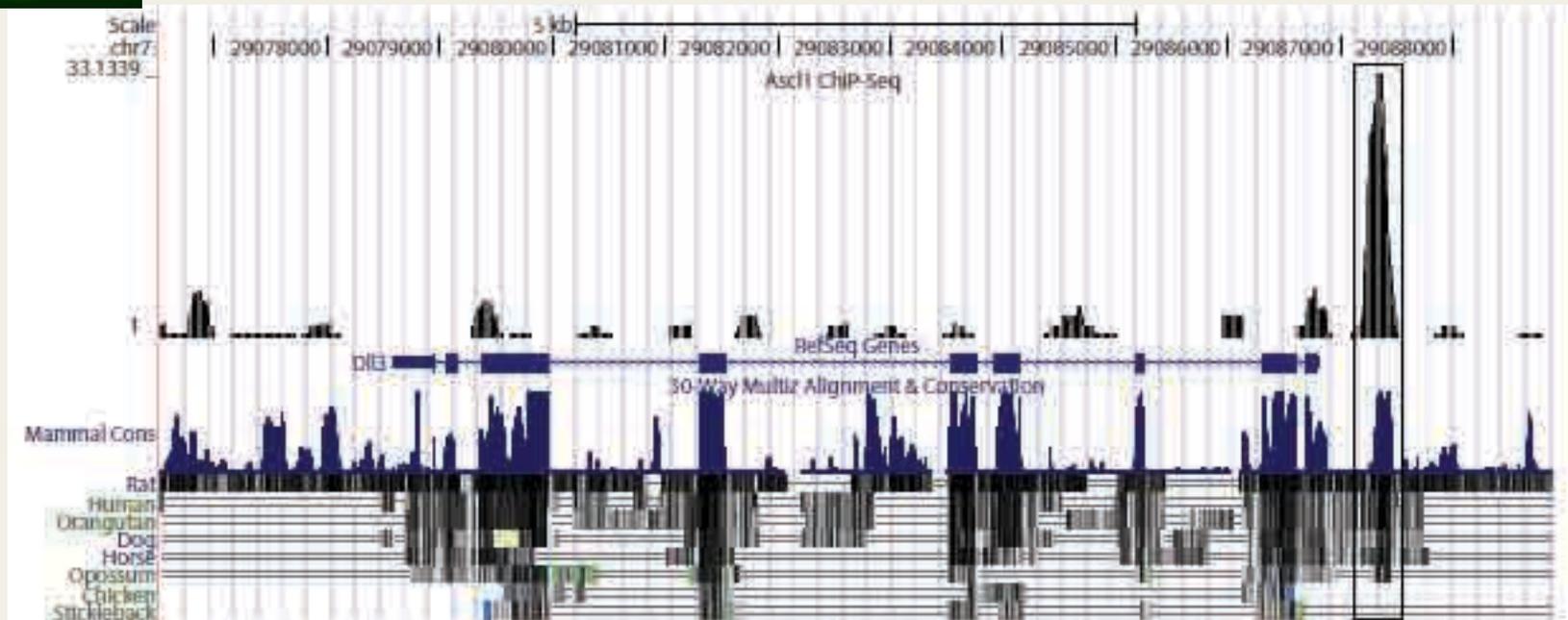


Library of transcription factors

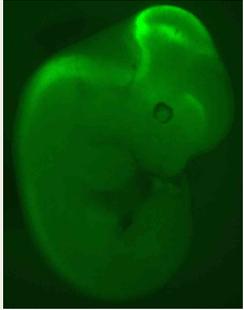




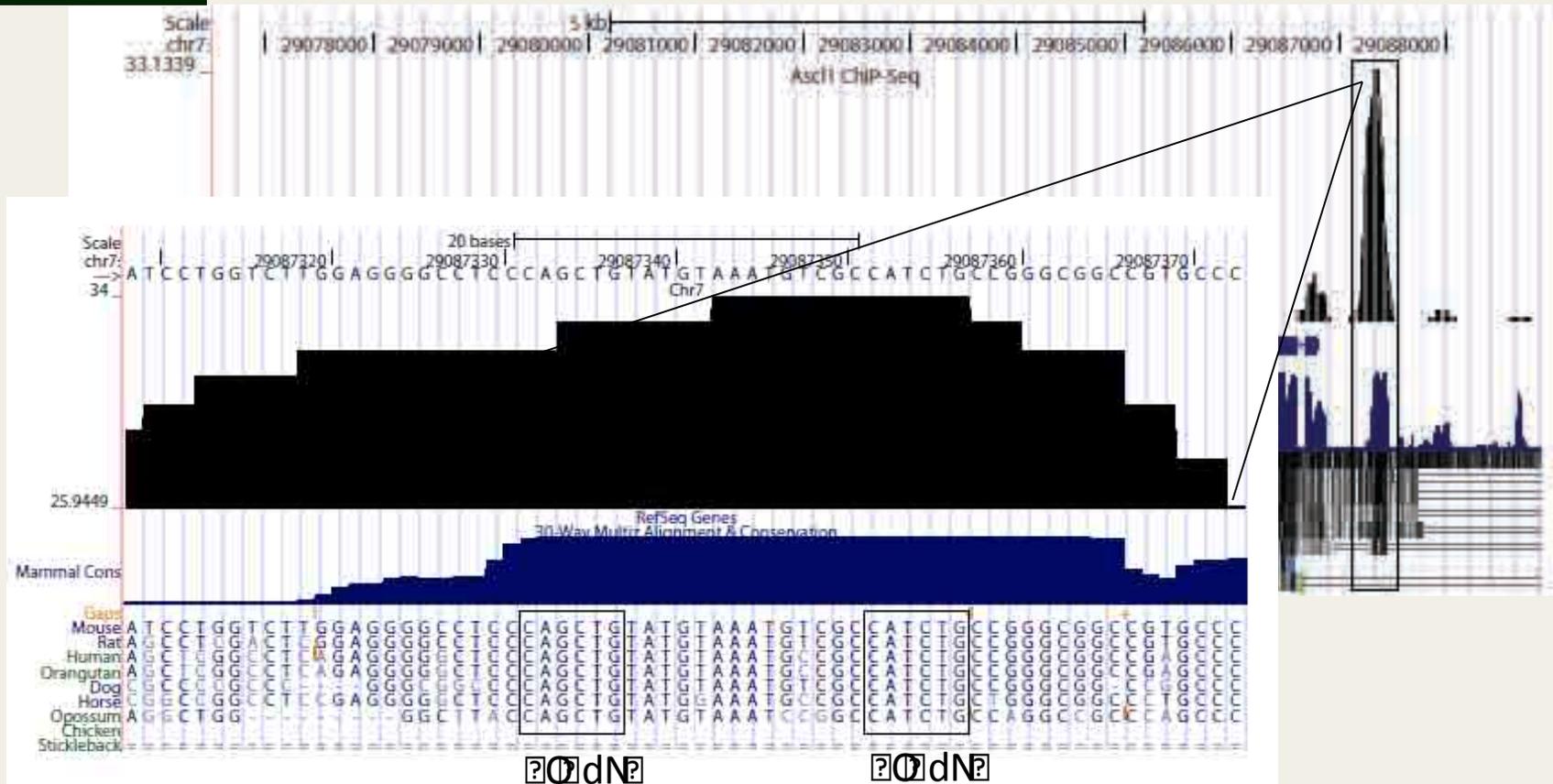
Ascl1 ChIP-Seq: Dll3



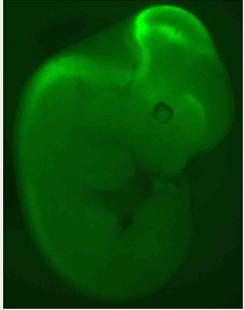
Window from UCSC Genome Browser with millions of 36 bp sequence reads from the ChIP-seq data mapped to the mouse genome. Ascl1 bound region is boxed and shows its location in the proximal promoter of Dll3.



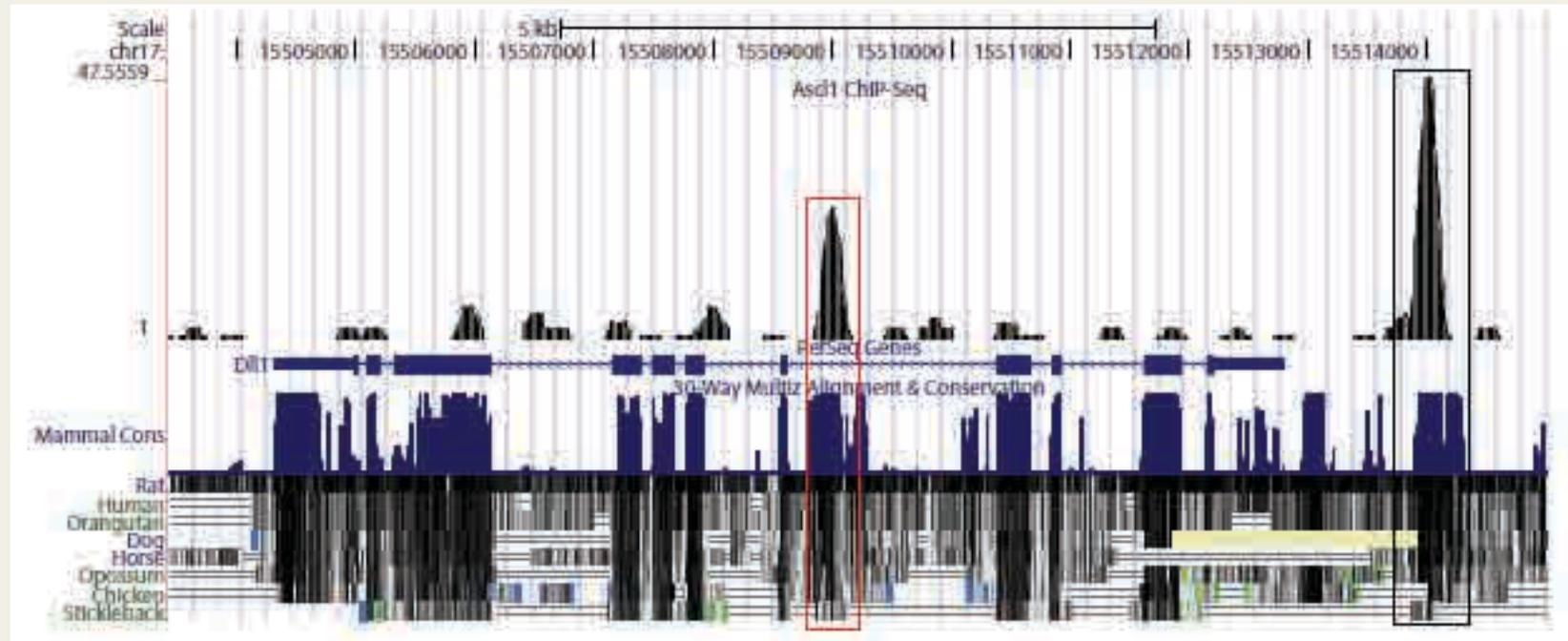
Ascl1 ChIP-Seq: Dll3



Using the USCS Genome Browser you can zoom in to see the sequence under the peak that has highly conserved Ascl1 binding sites.

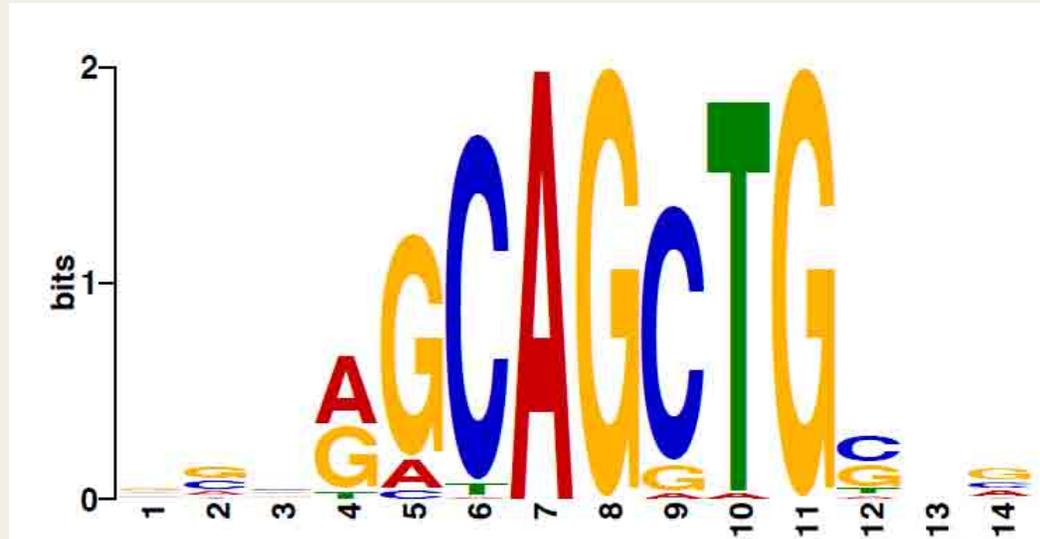


Ascl1 ChIP-Seq: Dll1



ChIP-seq data reveals a previously characterized *Ascl1* binding site in the *Dll1* promoter (black box) as well as an novel site within an intron of *Dll1* (red box).

De Novo Motif Analysis

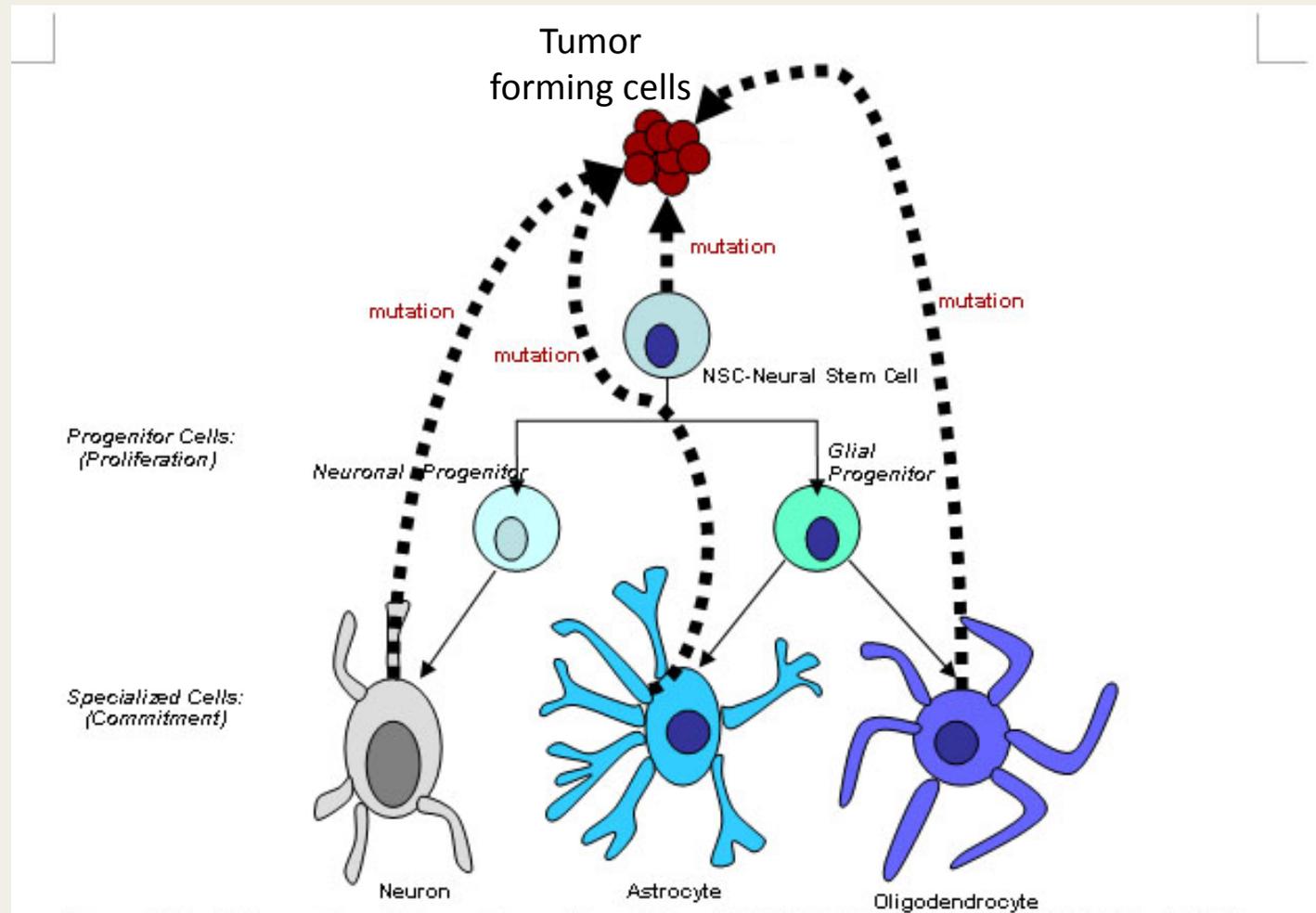


~2,000 high confidence binding sites called so many novel targets of *Ascl1* identified that can be studied for their function in neural development.

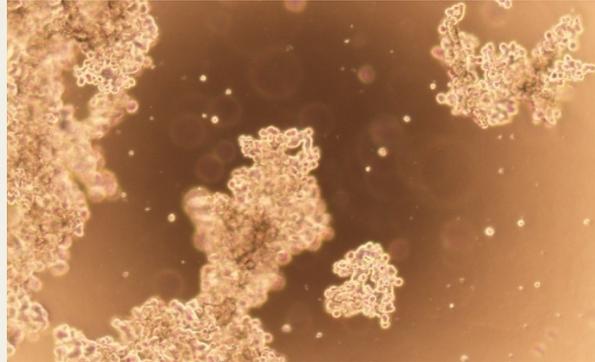
This motif matrix appeared in every peak and defines a more restricted recognition sequence for *Ascl1*.

Normal regulators of development show up in cancer cells

Ascl1 in glioblastoma and multiple neuroendocrine tumors

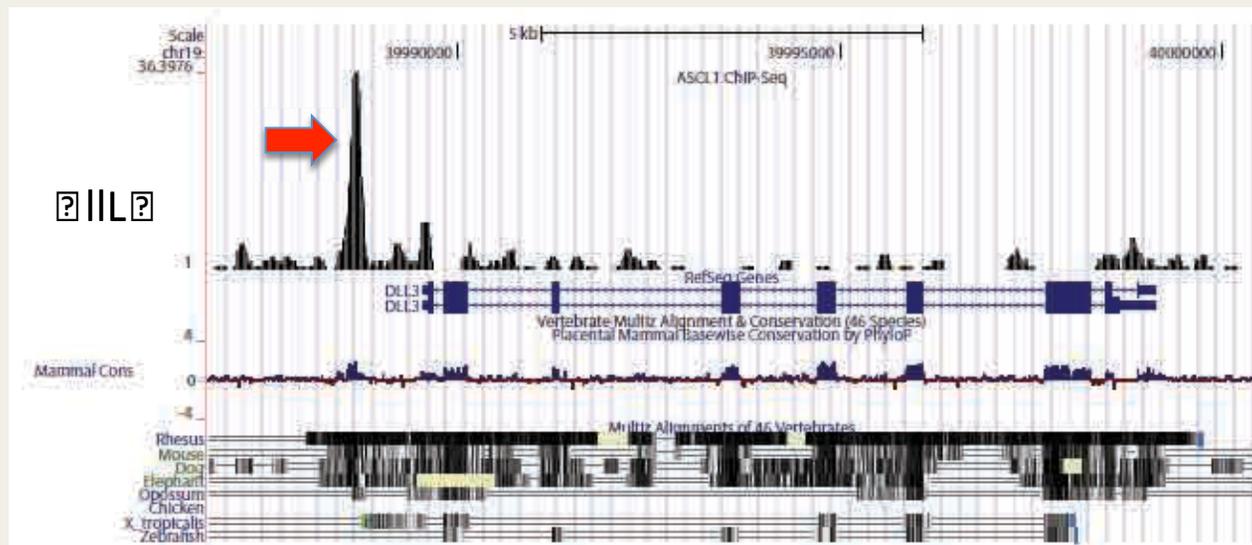
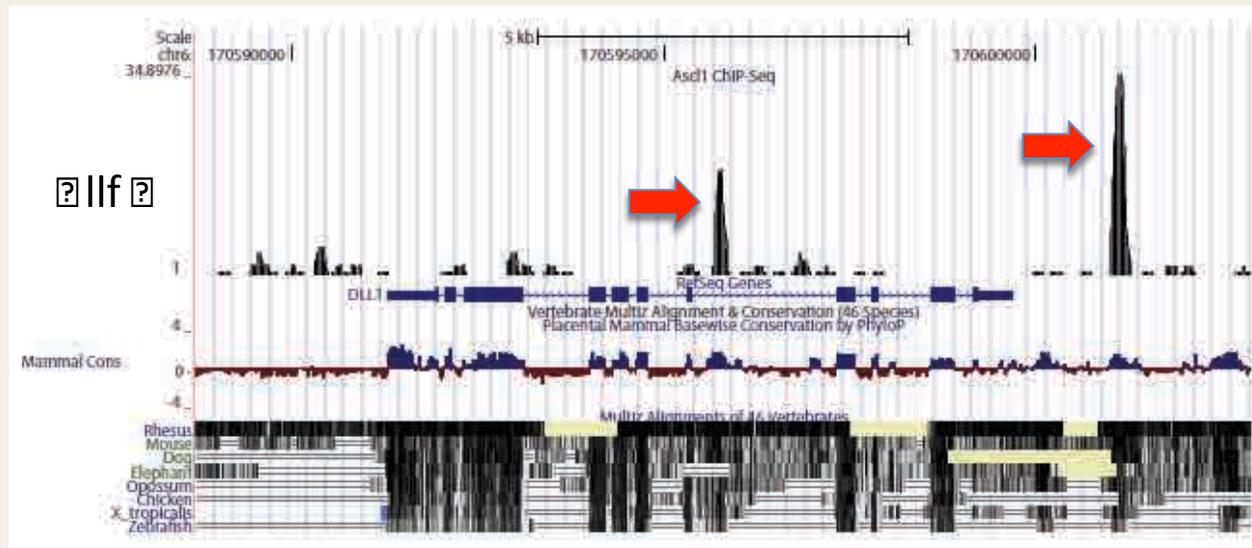


Small Cell Lung Cancer

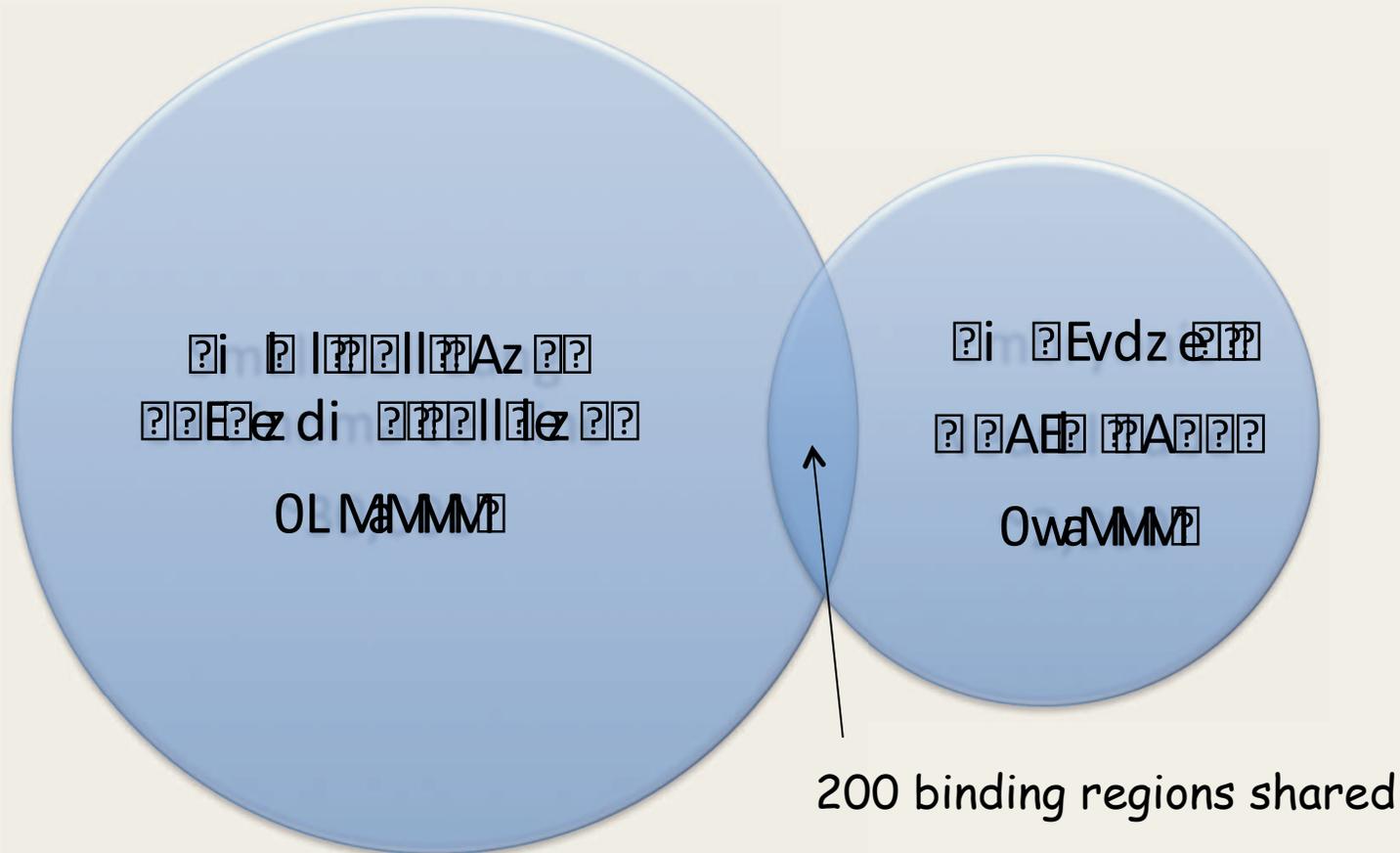


- ▶ *Ascl1* is highly expressed neuroendocrine cancers (Small cell lung cancer, medullary thyroid carcinoma, pheochromocytoma)
- ▶ ***Ascl1* likely contributes to the progressive development and behavior of the tumor cells but not the initial event.** Knocking down levels of *Ascl1* in tumor cell lines results in cell death and inhibition of growth.
- ▶ What are the transcriptional targets of *Ascl1* that explain the biological behavior and may be novel therapeutic targets?

ChIP-seq with Ascl1 in the Small Cell Lung Carcinoma cell lines reveals Ascl1 bound to some similar regions as has been identified in the developing neural tube ie Dll1 and Dll3.



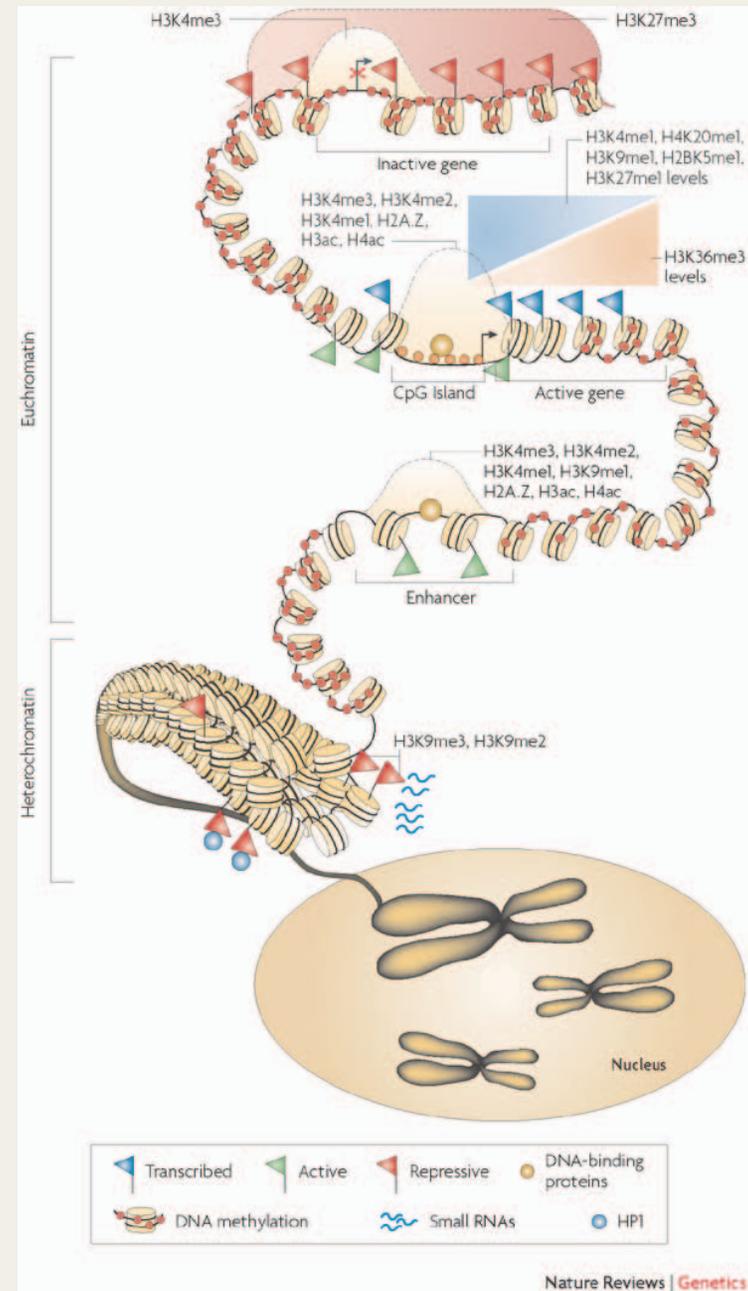
SCLC versus embryonic neural tube Ascl1 Binding Sites



Why such a difference in number and location?
Suspect epigenetic differences as major player.

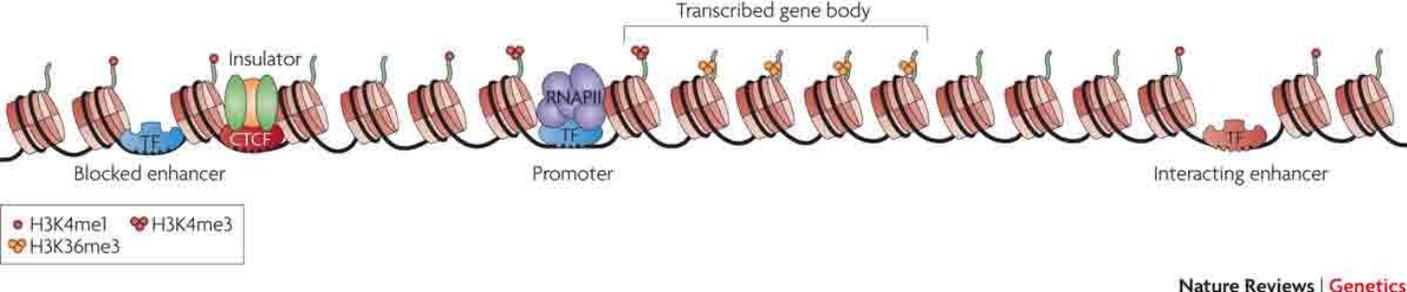
Transcription factor accessibility

- H3K4me3 and H3K27me3 are repressive marks
- H3K4me1 and H3K4me2 are active marks
- H3K9me3 and H3K9me2 are repressive marks

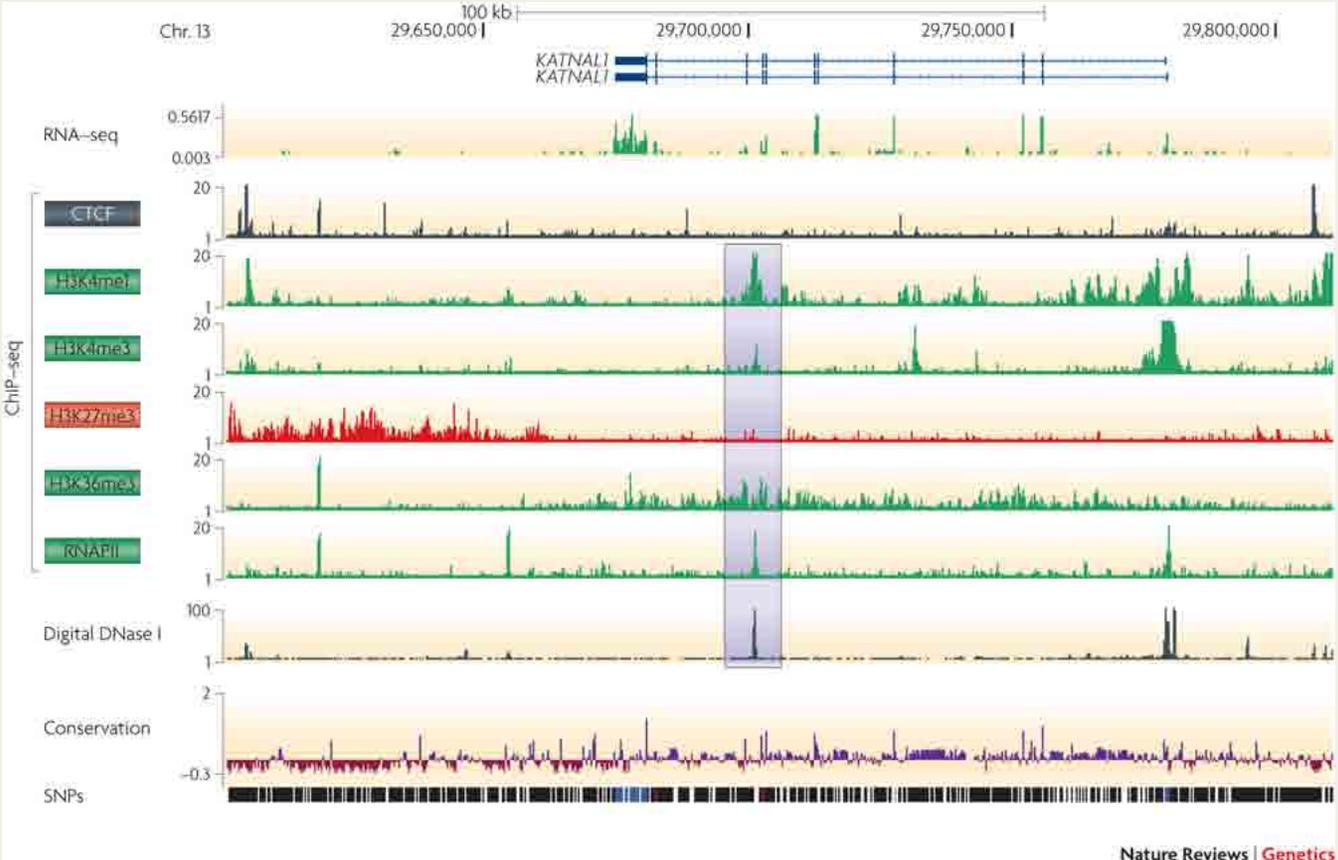


Any z p p F dz n s p r e F 0
 b A p p u e t n z y n d a
 f Z D f 3 2 2 F 3 W M H 2

Compare Ascl1 binding sites in the different tissues with respect to the epigenetic marks



Nature Reviews | Genetics



Nature Reviews | Genetics

Future

Coupling higher order chromatin changes with tissue specific factors to gain mechanistic insights into the transcriptional control of developmental processes.

Determine how these interactions change when things go awry in the cell as in cancer---leading to new therapeutic targets for treatment.

Strategies to harness the biological activities of transcription factors to direct differentiation of stem cells or reprogram cells to specific lineages. **i.e. recently shown that fibroblasts can be reprogrammed directly to neurons with Ascl1 being a critical component of the cocktail.**