Formation and patterning of the nervous system

II. Neural Patterning - patterning of neural progenitors along the dorsoventral axis
III. Neurogenesis - differentiation of neural progenitors into postmitotic neurons and glia.
IV. Understanding neural patterning in the context of neurogenesis

Overview of stages of neurogenesis

Organization of the spinal cord

Detection of proliferating cells in the CNS

The DNA content of a cell reveals its position in the cell cycle

Cells in S phase can be detected by their ability to incorporate labeled DNA precursors whose presence can be detected after labeling the cells (i.e. BrDU or 3H labeling - detection of proliferating cells in the CNS, birthdating neurons, cell fate).
Neurons are generated from mitotically active precursors and different types of neurons are generated in an orderly progression:

- VZ = ventricular zone
- IZ = intermediate zone
- PP = preplate
- SVG = subventricular zone
- SP = subplate
- CP = cortical plate
- MZ = marginal zone

The DNA analogs 3H-thymidine and Bromodeoxyuridine (BrdU) can be used to "birthdate" neurons. Cells that exit the cell cycle after labeling will be heavily labeled; cells that divide again dilute out the label. Incorporated into DNA during S-phase, but only available for a short time (then metabolized).

Cortical neurons are generated in an inside-first, outside-last order. What possible mechanisms might control the fates of cells produced at distinct times during development?

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Are cortical cells determined with respect to layer as they are generated or is their fate dictated from the position they migrate to?

How could one test these possibilities experimentally?

In the ferret layer6 cells are born in utero, weeks later cells are fated for layers 2 and 3 and must migrate through layers 6, 5 and 4 which are already formed.

Possible outcomes:

- Cells are multipotent; environment determines fate
- Cells are committed to a specific fate prior to migration

The behavior of early progenitors depends on their stage in the cell cycle at the time of transplantation:

1. Cells transplanted in S-phase change fates and become normal layer 2/3 neurons
The behavior of early progenitors depends on their stage in the cell cycle at the time of transplantation:

2. Cells transplanted after the S-to-G2 transition are committed layer 6 neurons.

Late progenitors are intrinsically and heritably committed to generating upper-layer neurons:

Transplant progenitors from older embryos into younger brains:

Early progenitors are multipotent: what about late progenitors?

What molecular mechanisms might control changes in progenitor cells over time?

1. First need to make more progenitors
2. To start to make neurons

...and glial!

Lateral inhibition and proneural genes
Each neuroblast emerges through a series of cell-cell interactions and changes in gene expression.

Identification of "proneural" mutations and Ac-Sc bHLH factors

Schematic representation of the structure of a bHLH dimer that is complexed to DNA. The basic region fits in the main groove of the DNA, and many residues in this region make direct contact with the DNA sequence. The two α-helices of both partners together form a four-helix bundle.

Functional specificities among proneural proteins. The functional specificities of the Drosophila proteins Scute (Sc) and Atonal, which are proneural factors for external sense organs and chordotonal organs, respectively, reside in residues that are located in the basic domain. Residues that differ between the basic regions of Scute and Atonal are predicted not to directly contact the DNA, but to be involved in interactions with cofactors. In the model, a cofactor interacts with both the basic motif of the proneural protein and the DNA sequence, and provides the proneural protein with specificity for binding to a particular E-box sequence.

Neurogenic genes in Drosophila: Notch, Delta, Enhancer of Split

Schematic illustration of the Notch and Delta genes products:

- Notch encodes a large transmembrane protein (300kD) that contains a large cytoplasmic domain, a single membrane-spanning segment, and a large amino-terminal extracellular domain (a receptor).
- Delta encodes a smaller homologue of Notch with nine EGF-like repeats in the extracellular domain. Genetic experiments suggested that Notch and Delta interact with one another and biochemical experiments showed that the interaction was direct.
- Enhancer of Split encodes for bHLH transcription factors, transcriptional readout of Notch signaling pathway.
Vertebrate homologues of Drosophila proneural and neurogenic genes

Notch signaling pathway

Vertebrate homologues of Drosophila proneural and neurogenic genes

Notch signaling underlies the process of lateral inhibition

Structure and properties of neural bHLH proteins. a. Dendrogram of the sequence of the basic helix–loop–helix (bHLH) domain of invertebrate (blue) and vertebrate (red) neural bHLH proteins. Proteins have been grouped according to the degree of sequence similarity in the bHLH domain. The proteins have been arranged on the left according to their position in the bHLH domain of proneural proteins, with neurogenin 2 (Ngn2) at the top. The proteins are grouped into two families on the right, with the two families further subdivided into subfamilies. The subfamilies are defined by the degree of sequence similarity in the bHLH domain of the proteins. Although neural bHLH proteins from different families recognize the common bHLH motif, they must recognize different motifs in the target promoters, as well as other regulatory elements.

FIGURE 1A ABA 20-kb cDNA expression in frog embryos during neural differentiation. In the images, the ontogenetic steps of the neural tube are indicated by the yellow arrow. The left side of the neural tube is stained with the neural-specific dye, while the right side of the neural tube is stained with the muscle-specific dye. This result indicates that the neural bHLH proteins are expressed in the neural tube, and that they are involved in the neural differentiation of neural plate stages, before the neural tube becomes differentiated.

FIGURE 1B ABA 20-kb cDNA expression in frog embryos during neural differentiation. In the images, the ontogenetic steps of the neural tube are indicated by the yellow arrow. The left side of the neural tube is stained with the neural-specific dye, while the right side of the neural tube is stained with the muscle-specific dye. This result indicates that the neural bHLH proteins are expressed in the neural tube, and that they are involved in the neural differentiation of neural plate stages, before the neural tube becomes differentiated.

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<tr>
<th>Animal</th>
<th>Gene</th>
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<tr>
<td>Mouse</td>
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Vertebrate proneural and neurogenic genes in neuronal differentiation

Proneural gene expression is induced at a high level in the neural progenitor, where it initiates a program that leads to neuronal differentiation. In neighboring cells, Notch signaling both represses and inhibits the activity of proneural genes, resulting in a block in differentiation. Proneural genes induce the expression of NeuroD, which promotes neuronal differentiation. In addition, vertebrate proneural genes promote cell cycle exit by inducing the expression of cyclin-dependent kinase inhibitors.

In parallel, vertebrate proneural genes also inhibit glial differentiation by blocking gliogenic signals.

Role of vertebrate proneural and neurogenic genes in glial differentiation - is glial a default state?

Generation of constitutively active Notch signaling by retroviral infection into the cortex.

FIGURE 1.31 Overexpression of the proneural AML1 gene NeuroD in frog embryos leads to the formation of a large number of ectopic neurons (right) all over the side of the animal. These neurons are never present in the normal animal (left). Neural tissue expresses the neural cell adhesion molecule (NCAM), and both embryos have been stained for this protein.

(From Lee et al., 1995)
Identification of neurogenin, a vertebrate neuronal determination gene.
Ma, Q., Kintner, C., and Anderson, D.J.
Cell, 87, Pgs. 43-52, 1996

At this point what is the historical basis of trying to identify bHLH factors?
MyoD – the master regulator of muscle development, is there an equivalent factor “i.e neural determination factor”??

How was the experiment done? (degenerate PCR)
Cloning of the Xenopus cDNA
Why try to identify additional bHLH factors besides Mash and NeuroD?
(preliminary exper with mouse neurogenin)

Figure 2. Sequential Expression of neurogenin and NeuroD in Rat Chick embryo

Figure 3. Sequential Expression of Neurogenin and NeuroD mRNAs in Chick

Figure 4. Induction of Endogenous Mash1 Expression and Neurogenin by Injection of A and C mRNA

Figure 5. X-CHX31 Induced Expression of Mash1 and NeuroD mRNA

Figure 6. Induction of Mash1 and NeuroD Expression by Site-directed Mutants of Mash1
NEUROGENIN BOTH ACTIVATES, AND IS INHIBITED BY, THE LATERAL INHIBITION MACHINERY

Figure 4. Model for the Role of KAGGF1 and Wnt5a in Lateral Inhibition and Neural Determinate