Major Events During Prenatal Development of the Cerebellum
(modified Fig. 9 from the Altman, 1982 review)

E14
The early-generated (day E13) deep nuclear neurons sojourn in a nuclear transitory zone in the superficial cerebellum prior to migration. The cerebellar neuroepithelium (CNEP) is still generating younger deep nuclear neurons and the oldest Purkinje neurons.

E16
Most of the deep nuclear neurons have left the CNEP and are still sojourning in the superficial nuclear transitory zone prior to migration. The Purkinje neurons are sojourning in the deep cortical transitory zone prior to migration. The CNEP is still generating the youngest Purkinje neurons and probably some glia and ependymal cells.

E17
Some of the deep nuclear neurons are leaving the superficial nuclear transitory zone and begin to settle in the deep nuclei. A few Purkinje neurons are leaving the deep cortical transitory zone, migrating upward through and between the deep neurons. The external germinal layer (egl) makes its first appearance in the posterior cerebellum, growing forward beneath the pia from a complex area called the germinal trigone (gt), where the CNEP, egl, and choroid plexus are joined. The CNEP is much thinner and is probably generating glia and ependymal cells.

E18
The egl continues to grow forward over the developing cerebellum. Beneath it, some Purkinje neurons settle in a thick layer, but they never extend in front of the advancing egl. Most Purkinje neurons are still migrating upward through the descending deep neurons. Most of the deep neurons are settling in the deep cerebellar white matter. The CNEP continues to thin and is probably generating glia and ependyma.

E20
The egl now covers the entire developing cerebellum above a thick layer of Purkinje neurons. Some Purkinje neurons are still migrating upward into the Purkinje layer. Most of the deep neurons are already settled in the deep cerebellar white matter. The CNEP continues to thin and is probably generating glia, ependyma, and some late-generated macroneurons, the Golgi cells.

- **Cerebellar neuroepithelium (CNEP) and ependyma**
- **External germinal layer (egl)**
- **Sojourning (cortical transitory zone) and primitive migrating Purkinje neurons**
- **Settling Purkinje neurons**
- **Sojourning (nuclear transitory zone) and primitive migrating deep nuclear neurons**
- **Settling deep nuclear neurons**
- **Unidentified fibers**
- **Germinal trigone (gt)**
The time of origin of macroneurons in the cerebellum and their afferents and efferents as determined by progressively delayed cumulative labelling with $^3$H-thymidine. The time span of the bar graphs is exact; the height of the bars is schematic. Tall bars indicate the days of peak production. **There is a sequential neurogenetic gradient in every group of graphs.** That pattern is probably essential for the development of a highly oriented cytoarchitectonics in the mature cerebellar cortex. (Modified figure 3 from the Altman, 1982 review).
Tracings of the Rat Midline Cerebellum (vermis) in the sagittal plane

- Outermost external germinal layer (proliferative zone) attached to pia
- Innermost external germinal layer (premigratory zone) intermingled with upper molecular layer
- Molecular layer (Purkinje cell layer is at internal border)
- Granular layer (Purkinje cell layer is at interface with molecular layer)
The concentration of heavily labeled cells in the lower half of the molecular layer (red line, the domain of basket cells) and the upper half (blue line, the domain of stellate cells) resulting from 4 consecutive daily injections of \(^3\)H-thymidine on the days indicated. Basket cells are generated earlier than stellate cells, peaks on postnatal days 4-7 compared to peaks on postnatal days 8-11. These data are not based on the delayed comprehensive cumulative labelling type of analysis. However, when these data are compared with those in modified Figure 12, it is clear that there is a strong sequential time of origin between cerebellar microneurons: basket cells are generated first, stellate cells next, and granule cells last. (Modified Figure 11 from the Altman, 1982 review.)
RELATIVE AGES OF GRANULE CELLS IN THE MIDLINE VERMIS

Vla ANTERIOR DECIVE
Vlb POSTERIOR DECIVE

V DORSAL CULMEN
IV VENTRAL CULMEN
III DORSAL CENTRALIS
II VENTRAL CENTRALIS
I LINGULA
X NODULUS

% labeled with 4 injections of ^3^H-thymidine on P16-P19

- 25-45%—the settling sites of the oldest granule cells
- 45-60%—the settling sites of middle-aged granule cells
- >60%—the settling sites of the youngest granule cells

The depths of the lobules (*) generally contain older granule cells than superficial parts (+). That may be related to the hypothesis that Purkinje cells in fissures become “tethered” to their output targets earlier than superficial Purkinje cells (Altman and Bayer, 1996). Another generalization is that the earliest maturing lobules are in the ventral vermis, the lingula (I) and nodulus (X). The latest maturing lobules are in the dorsal vermis behind the primary fissure, the posterior decline (Vlb), the tuber (VII), and the pyramid (VIII). When these data are compared with those in modified Figure 11, it is clear that granule cells have the longest time span of origin of the microneurons, and most are generated after the basket and stellate cell populations.

(Modified Figure 12 from the 1982 Altman review.)
The explanation for the unique shape of the Purkinje cell dendrite

Basket cell axons form a “tunnel” around the upward growing Purkinje cell dendrite and allow only one primary branch to grow toward the external germinal layer.

Stellate cell axons induce secondary branching of the Purkinje cell dendrite.

Bipolar cells are immature granule cells that grow the crossbar of the “T” part of their axon, a parallel fiber, before they migrate downward into the granular layer. The bipolar cells are always oriented parallel to the long axis of a particular cerebellar folium.

Altman’s “exclusion principle” limits the number of synapses with each parallel fiber and forces the spiny branchlets on the Purkinje cell dendrite to expand in the sagittal plane at a right angle to the pile of parallel fiber axons.
The top half of the dendrite is abnormal. When the external germinal layer regenerated, the parallel fibers grew perpendicular to the normal orientation. As the Purkinje cell grew its dendrite among the flipped parallel fibers, its planar part “flipped” and produced secondary branches with spiny branchlets fanning out in the coronal plane, exactly perpendicular to the stack of abnormally oriented parallel fibers.

This tells us something else:
A reconstituted external germinal layer loses the ability to normally orient itself so that parallel fibers always grow perpendicular to the long axis of a given cerebellar folium.
**HOW INGROWING MOSSY FIBERS DETERMINE THE POSITION OF THE GRANULAR LAYER**

**NORMAL DEVELOPMENT**
In the normal cerebellum, granule cell precursors in the external germinal layer generate young neurons in specific time periods and meet the ingrowing mossy fibers in the granular layer.

- Granule cell precursors proliferate in the external germinal layer
- Young granule cells migrate downward leaving their axons, the parallel fibers, in the molecular layer
- Ascending mossy fibers (axon terminals of neurons from the precerebellar nuclei in the medulla and pons)

**ABNORMAL DEVELOPMENT**
In the abnormal cerebellum where X-ray schedules kill the precursors of granule cells, the reconstituted external germinal layer **delays granule cell migration**. As the mossy fibers continue to grow upward, they arrest the young granule cells in an ectopic position in the molecular layer.

- X-rays kill nearly all EGL cells
- The few surviving cells slowly regenerate a new EGL
- External germinal layer
- Molecular layer (with ectopic granule cells)
- Purkinje cell layer
- Medullary layer