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# Peri- and Postnatal Development of Cerebellar Compartments in the Mouse

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**Abstract** The adult cerebellar cortex is compartmentalized into longitudinal stripes, in which Purkinje cells (PCs) have compartment-specific molecular expression profiles. Since the striped compartments have specific afferent and efferent projection patterns, they underlie the functional localization of the cerebellum. How these compartments form during development is generally not understood. Our recent study focuses on development of the cerebellar compartmentalization from embryonic day 17.5 (E17.5), when embryonic clustered compartmentalization is evidently observed, to postnatal day 6 (P6), when adult-type striped compartmentalization begins to be established, in mouse. FoxP2, one of the marker molecules for immature PCs, has been used to identify E17.5 PCs. PC subsets or clusters have been distinguished from each other by using different expression profiles of several marker molecules (PLCβ4, EphA4, Pcdh10, and a reporter molecule of the 1NM13 transgenic mouse strain). Analysis of spatial organization of PC clusters by three-dimensional reconstruction from multiple-stained serial sections has indicated 54 PC clusters in the E17.5 cerebellum. Individual clusters are spatially rearranged into stripes in the period from E17.5 to P6. In summary, the clustered compartments in the E17.5 cerebellum are basically direct origin of the adult-type striped compartments in the cerebellar cortex.

**Keywords** Purkinje cells · Zebrin II · Aldolase C · FoxP2 · Clusters

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## Introduction

The adult cerebellar cortex is subdivided transversely by its lobular folding and longitudinally by compartments of Purkinje cell (PC) subsets that are defined by the expression patterns of certain molecules. For example, in adulthood, aldolase C [1] or zebrin II [2] is highly expressed in PCs in 20–40 longitudinally arranged compartments [3, 4]. Since longitudinal compartments have specific projection patterns of efferent PC axons and afferent olivocerebellar axons [3–5], they represent some aspects of functional localization of the cerebellum.

The cerebellar compartmentalization and compartment-specific axonal projections are established during development. The immature PC layer is compartmentalized into several aggregations of PCs in the late embryonic period [6]. The compartments of aggregated PCs have different expression profiles of certain genes and molecules [7, 8]. Embryonic molecular compartmentalization seems to be essential for the establishment of functional cerebellar organization. The details of how the embryonic cerebellar compartments are organized in the late embryonic stage (at embryonic day 17.5 (E17.5)) and how they are transformed to the adult compartments have been recently studied in our laboratory [9]. This article is aimed at introducing this study and related backgrounds.

## PC Clusters in the E17.5 Mouse Cerebellar Cortex

To clarify compartmental organization of PCs in the entire embryonic cerebellum, one has to distinguish PCs from other cells, which is generally not easy unless PCs are specifically labeled. Among marker molecules to label embryonic PCs (RORα, Corl2, FoxP2), FoxP2 is particularly useful since it has some difference in expression intensity among different PC subsets [9, 10].

To identify clusters of the E17.5 PC layer, we have looked at labeling patterns of following marker molecules of PC subsets. PLCβ4 [11], EphA4 [12], and Pcdh10 are expressed only in particular PC subsets at E17.5 and hence they are useful in distinguishing PC subsets [10]. We have also used 1NM13 transgenic mouse strain [13], in which β-galactosidase is expressed in particular PC subsets after E14.5. In serial sections of the E17.5 cerebellum, PC clusters that have different expression profiles of these marker molecules, are distinguished by visualizing every combination of one (or two) of these compartmental markers and the general PC marker, FoxP2 (Fig. 1a). Adjacent PC clusters are often separated by a space (PC gap), which also helps to distinguish PC clusters.

The shape of the cerebellum is already complex at E17.5 (lobular distinction being formed and lateral hemispheral parts protruding rostrally). Therefore, to clarify the spatial organization of PC clusters in the whole E17.5 cerebellum, we have reconstructed all of the identified clusters in 3D space. We have depicted the contours of identified clusters on every serial coronal and horizontal section of the cerebella. We then have imported the drawings to 3D graphics software to reconstruct clusters. Immature major cerebellar fissures, which have been identified by carefully tracing in serial sections, have been also added to the scheme. In the resultant 3D reconstruction (Fig. 1b), we have recognized a total of 54 PC clusters. They are located at specific places, have different shapes, and have distinctive molecular expression profiles [9].

## PC Compartmentalization in the P6 Mouse

Following the clustered compartmental organization of the PC layer from E17.5 to postnatal day 6 (P6) has shown that the PC layer become single-cell thick and foliation of all major lobules became recognizable at P6. Each of the marker molecules (FoxP2, PLCβ4, EphA4, and Pcdh10) is still expressed in roughly the same PC subsets at P6 as they do at E17.5. Concerning β-galactosidase expression in 1NM13 strain, the final expression pattern emerges at P6 after additional expression that occurs in some PC subsets between E17.5 and P6

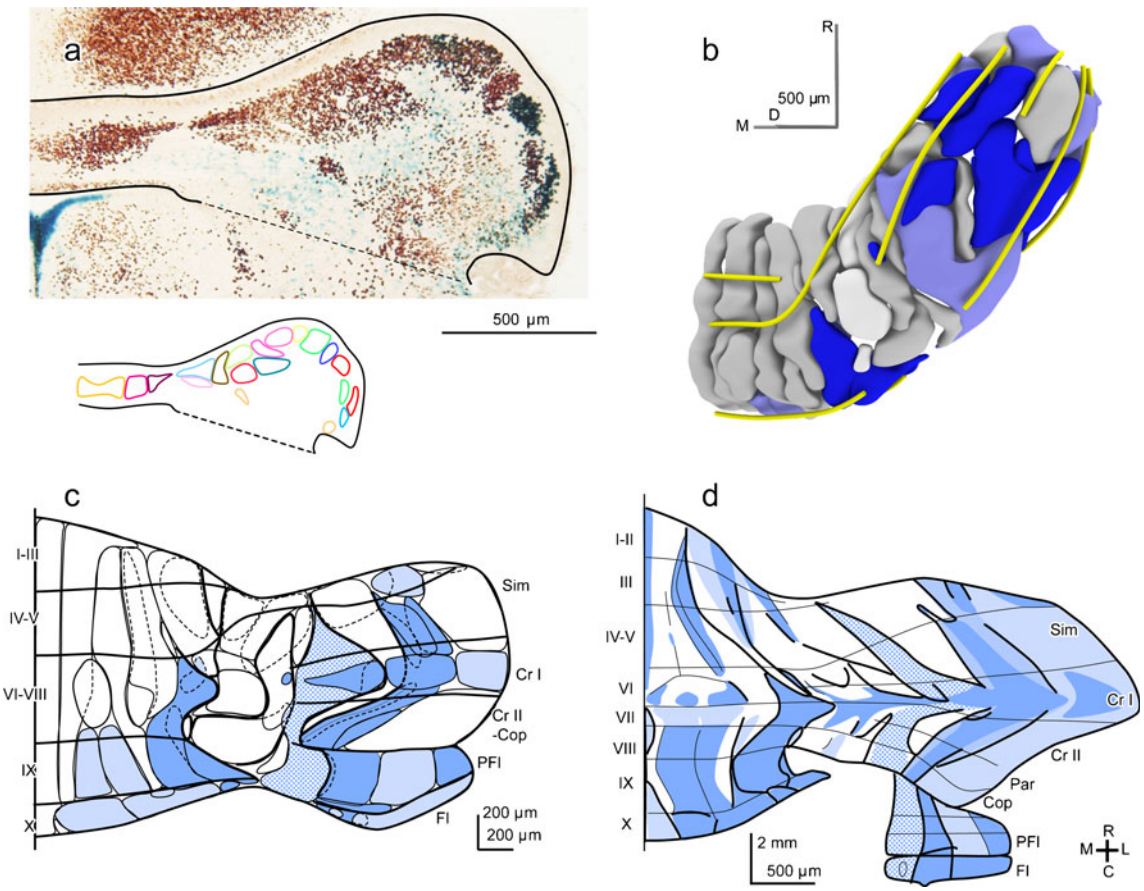


**Fig. 1** PC clusters in the E17.5 cerebellum. **a** Photomicrographs of double labeling of FoxP2 (*brown*) and β-galactosidase (*blue*) in a coronal section of the 1NM13 mouse cerebellum. Bottom inset shows contours of the individual clusters that were identified by referring to labeling for PLCβ4, EphA4, and Pcdh10 in addition. **b** Three-dimensional display (*dorsal view*) of PC clusters in the right E17.5 cerebellar cortex. **c**, **d** Schemes of spatial arrangements of PC clusters at E17.5 (**c**) and striped compartments of the PC layer at P6 (**d**) drawn on the unfolded scheme of the cerebellar cortex. Panels are based on data obtained in our research [9]

[13]. In addition, granule cell (GC) raphes, which are narrow PC-free spaces filled with GCs in the PC layer, is observed to subdivide the PC layer into multiple divisions at P6 [14] but not in adult. Stripe-shaped expression patterns of the marker molecules and their relation to GC raphes have been clarified in the P6 cerebellar cortex (Fig. 1d).

## Rearrangement of E17.5 PC Clusters into P6 PC Stripes

The organizations of the E17.5 and P6 cerebellar cortex are clearly different. The PC layer is thick and composed of clusters of PCs, some of which are located beneath others (earlier section), at E17.5. At P0 and then at P1, clusters become generally thinner and less frequently located beneath other clusters, which seems to be caused by the tangential rearrangement of PC clusters. Some clusters that are located beneath other clusters migrate laterally and upward to completely face the surface of the PC layer ("transverse slide"). Then, accompanied by longitudinal expansion of the PC layer or the cerebellar cortex and foliation of the cerebellar cortex, the PC layer becomes more flattened and clusters become more aligned on a single plane in the PC layer at P1 than at E17.5. This process represents the main developmental transformation of the PC layer during this period.

Following individual PC clusters in all immature lobules of the cerebellum between E17.5 and P6, by utilizing generally consistent expression patterns of the marker molecules, has indicated that the compartmental organization of PC subsets is almost the same between E17.5 and P6 (Fig. 1c and d). Individual clusters of PC subsets at E17.5 are basically transformed to individual stripes of PC subsets at P6. Furthermore, PC gaps at E17.5 seem to be all transformed to GC raphes at P6.

## Summary

The embryonic PC layer already has fine compartmentalization into about 50 clusters of PCs at E17.5. Individual E17.5 clusters basically developed into individual striped compartments by P6 through a rearrangement of PC clusters. Individual P6 compartments have not yet fully related to adult aldolase C (zebrin II) compartments, since aldolase C is not yet expressed at P6. However, it is likely that there is nearly a one-to-one relationship between individual P6 and adult compartments, since the final β-galactosidase expression pattern in 1NM13 mice is similar to the adult aldolase C expression pattern and the expression pattern of PLCβ4 is complementary to the adult aldolase C expression pattern [15]. In sum, the fine compartmentalization of the adult cerebellum is basically established as early as at E17.5. However, it still remains unclear how and when the PC compartments are formed before E17.5.

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