Early Purkinje Cell Development and the Origins of Cerebellar Patterning

Filippo Casoni, Laura Croci, Ottavio Cremona, Richard Hawkes, and G. Giacomo Consalez

Abstract This chapter explores the mechanisms that regulate Purkinje cell neurogenesis, revealing the finely timed contribution of many regulatory genes in the control of PC progenitor specification, proliferation, subtype differentiation, migration, and survival from the cerebellar primordium to the end of prenatal embryogenesis, discussing some of the key molecules involved and the ways they combine to generate the complex adult cerebellar architecture.

Keywords Zebrin • Transverse zone • Stripe • Ventricular zone • Ebf2 • Reelin

PCs as Project Managers of Cerebellar Cytoarchitecture and Connectivity

The cerebellum contains a limited number of cellular phenotypes, arranged in a highly conserved circuitry and identified by their morphological features, their reciprocal relationships, and the expression of distinctive neurochemical markers. The mouse is the main model system in which cerebellar ontogenesis has been studied extensively. Although the mammalian cerebellum is superficially homogeneous, it actually consists of several hundred distinct compartments, which form a complex, reproducible array of transverse zones and parasagittal stripes. Cerebellar architecture is built around multiple Purkinje cell subtypes [1–6] – most notably zebrin II/

F. Casoni • O. Cremona • G.G. Consalez (✉)
Università Vita-Salute San Raffaele, Via Olgettina 58, 20132 Milan, Italy

Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy
e-mail: g.consalez@hsr.it

L. Croci
Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

R. Hawkes (✉)
Department of Cell Biology and Anatomy, and Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada
e-mail: rhawkes@ucalgary.ca

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aldolase C [7, 8] – which form the transverse zone-and-parasagittal stripe scaffold upon which the adult cerebellum is built. For example, zone-and-stripe boundaries restrict the terminal fields of many cerebellar afferent projections (reviewed in [9]), interneuron neurites [10] and somata (reviewed in [11]), and glial gene expression profiles (e.g., 5′-nucleotidase [12]).

In the mouse, the general timeline of events that leads to cerebellar maturation from its embryonic *anlage* has been fully clarified [13–18]. Here we discuss some of the major features of cerebellar development, focusing on the ontogenesis of Purkinje cells (PCs), the sole projection neurons of the cerebellar cortex.

PC development is only partially characterized, despite the remarkable progress made in recent years (reviewed in [19]). Achieving a better understanding of PC cell fate specification and ontogenesis in general is important for a number of reasons. First, PCs orchestrate the early stages of cerebellar development, namely those that precede the massive proliferation of granule cell precursors in the external granular layer. Only later in embryogenesis, and especially after birth, do granule cells take control of cerebellar histogenesis and foliation, as they outnumber all other cerebellar cell types by several orders of magnitude.

Secondly, PCs actually control granule cell clonal expansion by releasing the extracellular morphogen/mitogen sonic hedgehog [20–23], with the result that the overall PC number heavily influences the final dimensions and organization of the cerebellum – and ultimately its function. The corollary is that defective PC genesis or migration impairs granule cell clonal expansion, and cerebellar foliation/PC migration failures result in a lissiform adult cerebellar cortex: e.g., the naturally occurring mouse mutant *reeler* (Relnrl: reviewed in [24]).

Thirdly, PCs guide the wiring of the cerebellum. Most afferent fiber systems invade the cerebellum at around embryonic day 13/14 (E13/14) in the mouse [25, 26] and terminate with a spatial organization that parallels the pattern of PC stripes [27]. PCs instruct afferent fibers, including olivocerebellar axons, which eventually establish a one-to-one contact with their target, as well as mossy fibers, which connect transiently with PCs and use PC-produced guidance cues prior to retracting and shaping their definitive synapses on granule cell dendrites (reviewed in [16, 28]). PC subtype organization is thought to play a key role in instructing circuit wiring into topographic maps: zone-and-stripe boundaries typically restrict the terminal fields of both cerebellar mossy fiber and climbing fiber afferent projections (reviewed in [9]), and interneuron neurites ([10], reviewed in [11]) and spontaneous and engineered mouse mutants with disrupted PC stripes have complementary alterations in the spatial arrangement of afferent terminals [29–31].

Cerebellar Anlagen and Germinal Zones

The cerebellum arises from a specialized region at the midbrain/hindbrain boundary [32–34]. In the mouse, at E8.5, the antagonistic interaction that takes place between homeobox genes *Otx2* and *Gbx2* defines the isthmic organizer region [35, 36], which controls the development of cerebellar structures via the secreted

morphogens FGF8 and WNT1 [16, 37, 38]. At this stage, the cerebellar primordium consists of two distinct and symmetric bulges thought to grow and fuse on the midline, eventually giving rise to the vermis, flanked by the two hemispheres [15]. Importantly, however, homotopic and isochronic quail-chick grafting experiments have clearly shown that the caudal part of the early midbrain vesicle generates the rostral and medial part of the prospective cerebellum [32, 39–42]. Thus, the anterior part of the prospective cerebellar vermis, instead of resulting from fusion of lateral cerebellar plates (His, 1889), likely originates from the caudal and alar portion of the mesencephalic vesicle [39].

Once a low-resolution map has been drawn, cerebellar histogenesis begins, starting at E9. Around E9.5, two germinal neuroepithelia emerge in the cerebellar primordium, abutting the opening of the fourth ventricle: (1) the rhombic lip (RL), located at the outer aspect of the cerebellar plate, adjacent to the roof plate (RP, dorsal), and (2) the ventricular zone (VZ), lining the lumen of the fourth ventricle (ventral). These stem cell/progenitor compartments may be identified by the region-specific expression of two genes encoding basic helix-loop-helix transcription factors: pancreas transcription factor 1a (*Ptf1a*) in the VZ [43], and atonal homolog 1 (*Atoh1*) in the RL [44]. Cerebellar radial glial progenitors [45] expressing *Ptf1a* are fated to generate all GABAergic neurons of the cerebellum, including PCs and all inhibitory interneurons – cerebellar nuclear interneurons plus basket, stellate, Golgi, and Lugaro cells in the cerebellar cortex [43, 46, 47]. Homozygous mutations of *PTF1A* are associated with cerebellar agenesis in humans [48]. Conversely, all glutamatergic lineages – the large projection neurons of the cerebellar nuclei, unipolar brush cells, and granule cells – derive from *Atoh1*$^{+}$ progenitors [49–54]: their development is exhaustively reviewed elsewhere [19].

Important genetic networks involved in the maintenance of the stem cell/progenitor pool and in cell fate specification are active in the VZ and/or RL between E10 and E13. The stem cell marker SOX2 is expressed in both neurogenic territories (VZ and RL) and in the RP [55]. Its homolog SOX9 is largely co-expressed with SOX2 and may mediate termination of neurogenesis, thereby regulating a neurogenic-to-gliogenic fate switch in the mouse cerebellar primordium [55]. The target of Notch signaling, *Hes5*, is expressed in the VZ and RL, with a very sharp boundary and no expression in the RP. However, *Hes1* expression levels are low to absent in the VZ and RL but present in the RP [56, 57]. Notch1 in the cerebellar primordium interferes with BMP2/BMP4 signal transduction causing downregulation of the BMP target *Msx2*.

As shown by birthdating studies, cerebellar projection neurons, (PCs in the cerebellar cortex and glutamatergic neurons in the cerebellar nuclei), are born first, at the outset of cerebellar neurogenesis, while both inhibitory and excitatory interneurons are generated perinatally [15, 58, 59]. Dividing VZ precursors delaminate into the cerebellar presumptive white matter, while those of the RL migrate below the pial surface where they form the rhombic lip migratory stream, initially containing nucleofugal neuron progenitors and, later, the granule cell precursors of the external granular layer. Postnatal neurogenesis continues in both regions through the third postnatal week, giving rise to GABAergic and glutamatergic interneurons, respectively [15, 17].

Establishment of Neurogenic Microdomains for GABAergic Progenitors

A schematic representation of microdomains present in the cerebellar VZ is provided in Fig. 1. All cerebellar GABAergic neurons originate in the VZ from *Ptf1a*$^+$ [43] and *Ascl1*$^+$ [60] progenitors according to a two-step sequence [17, 19]. First, projection neurons (nucleo-olivary neurons and PCs) are generated from stem cells that give rise to fate-committed precursor populations. The nucleo-olivary neurons are generated between E10.5 and E12.5 in the mouse. Next, starting around E11 and through E13.5, mitotic PC progenitors exit the cell cycle and layer on top of the VZ

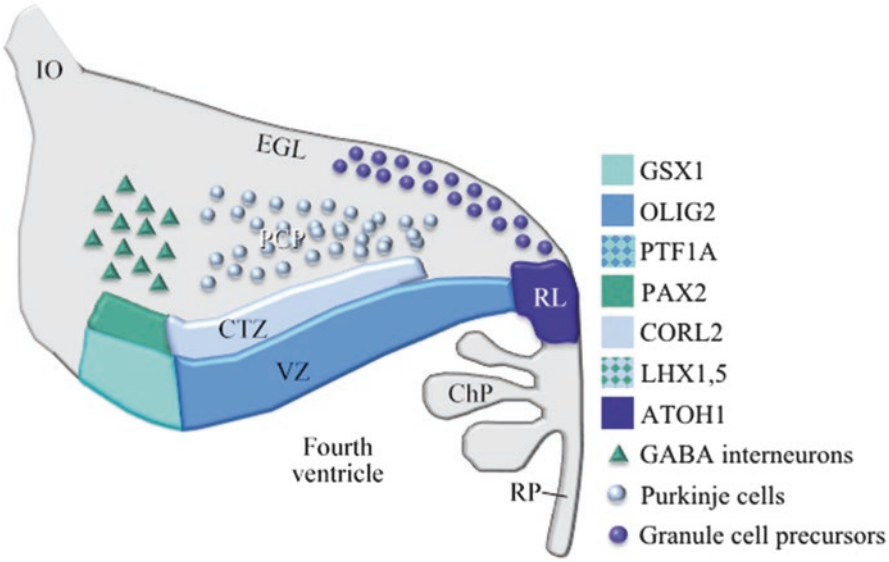

Fig. 1 A simplified representation of gene expression and cellular domains present in the E12.5 murine cerebellar primordium and giving rise to the mature cerebellar cortex. No reference is made here to cerebellar nuclei and their precurosrs. The drawing represents a sagittal section of the cerebellar anlage. The cerebellar primordium is bordered by the isthmic organizer (*IO*) rostrally and by the roof plate (*RP*) caudally. The choroid plexus (*ChP*), a roof plate derivative, is also shown. RP and ChP are non-neurogenic territories. The ventricular zone (*VZ*) is a mitotic cellular domain abutting the lumen of the fourth ventricle and giving rise to all GABAergic neurons of the cerebellar cortex. PTF1A is expressed by all GABAergic progenitors of the VZ, including PCs and interneurons. The PTF1A domain contains GSX1$^+$ cells (mitotic interneuron progenitors) and OLIG2$^+$ cells (mitotic PC progenitors). Both populations delaminate from the VZ (see text) giving rise to subventricular domains. The cortical transitory zone (CTZ) contains CORL2$^+$ potmitotic PC precursors that subsequently migrate into the Purkinje cell plate (PCP), underneath the external granular layer (EGL). GSX1$^+$ interneuron progenitors delaminate and give rise to PAX2$^+$ interneuron precursors fated to populate the prospective white matter (not shown) before homing into the cortex. Both PAX2$^+$ and CORL2$^+$ domains are also positive for LHX1 and LHX5. Finally, all glutamatergic neurons of the cerebellum originate from the rhombic lip (RL, positive for the proneural gene *Atoh1*). Among them, granule cell precursors migrate tangentially beneath the pia mater and populate the prospective EGL

to populate the nascent PC plate. The GABAergic interneurons are first born around E11 and sequentially generate all inhibitory local circuit neurons of the mature cerebellum.

The VZ is subdivided into mitotic progenitor domains abutting the ventricular lumen and corresponding postmitotic domains in the cerebellar primordium (an additional microdomain defines the rhombic lip) [61]. A microdomain positive for PTF1A contains two genetically defined progenitor cell types: OLIG2$^+$ PC progenitors occupy a more caudal position and undergo their terminal mitosis between E11 and E13; GSX1$^+$ progenitors are located more rostrally and medially. At E12.5, corresponding to the peak of PC neurogenesis, the c2 territory can be subdivided into a more caudal microdomain positive for CORL2, a selective marker of postmitotic PC precursors, and into a rostral/medial microdomain containing PAX2$^+$ interneuron precursors. PC precursors, after leaving the cell cycle, start migrating and populate different regions of the cerebellar cortex according to their birthdate [15, 52]. Instead, actively proliferating interneuron progenitors (IPs), positive for GSX1, begin to delaminate from the VZ giving rise to PAX2$^+$ interneuron progenitors and then migrate in successive waves to the nascent cerebellar nuclei or, with an inside-out progression, to the granular and molecular layers of the cerebellar cortex, where they acquire their definitive identities under the influence of instructive environmental cues ([62], reviewed in [63]).

The Regulation of PC Progenitor Specification and Commitment

At early stages (E11–12.5), a small number of GSX1$^+$ interneuron precursors are found in the most rostral region of the VZ, while the majority of PC progenitors occupy more caudal regions of the VZ. Ablation of *Olig2* has only a small effect [64] or no effect on PC number. However, a null mutation of both *Olig2* and *Olig1* produces a reduction of committed PC precursors [46]. As development proceeds, PC progenitors progressively become interneuron precursors, which spread from rostral (close to the isthmic organizer) to caudal, at the boundary between RL and RP. This temporal identity transition of cerebellar GABAergic neuron progenitors from PC progenitors to interneuron precursors is negatively regulated by OLIG2 and positively by GSX1 [46]. However, this view is challenged by the results of short- and long-term lineage tracing studies performed by other authors [64], suggesting that *Olig2*$^+$ progenitors may not contribute importantly to the interneuron precursor lineage. Further analyses will be required to resolve this discrepancy: one possible scenario is that *Gsx1*$^+$ progenitors affect the number of PC-committed *Olig2*$^+$ precursors (or the maintenance of the PC-committed stem cell pool) through a paracrine, non-cell-autonomous mechanism.

The VZ subregion containing PC progenitors is also characterized by the strong expression of E-cadherin (encoded by *Cdh1*) and of the cell surface marker NEPH3, which is a direct downstream target gene of PTF1a [65]. When OLIG2$^+$ PC progenitors

exit the cell cycle, they activate the expression of *Corl2* [66], which encodes a transcriptional repressor [67], and that of *Lhx1* and *Lhx5* [68], encoding LIM homeobox domain proteins. However, unlike CORL2, LHX1 and LHX5 label delaminating interneuron precursors as well as postmitotic PC precursors [46, 66]. Cells co-expressing LHX1/LHX5 [68] and CORL2 [66] are *bona fide* differentiating VZ-born precursors committed to a PC fate.

Other PTF1A targets are expressed in the VZ in addition to those described above [69]. The *Drosophila atonal* homologs *neurogenin 1* and *neurogenin 2* are proneural genes encoding basic helix-loop-helix transcription factors. *Neurog1*$^{+}$ progenitors give rise to inhibitory cortical interneurons and some PCs [70, 71], while *Neurog2* is expressed mainly in the PC- and presumptive nucleo-olivary neuron lineages. NEUROG2 controls progenitor cell cycle progression, promotes cell cycle exit and differentiation, and spurs the cell-autonomous phase of PC precursor dendritogenesis. Nullisomy for *Neurog2* causes a reduction in the overall PC number [72]. However, NEUROG1 and NEUROG2 are not required for the adoption of a PC fate (R. Hawkes, unpublished observation, and [72]). Interestingly, cell cycle analysis conducted by cumulative S-phase labeling on *Neurog2*^{CreERT2} knockin mice has revealed for the first time that at the peak of PC neurogenesis (E12.5), dividing VZ progenitors cycle in ∼14 h, and their basal-to-apical oscillating motion is compatible with interkinetic nuclear migration, similar to what has been shown in other territories of the neural tube, but never before in the cerebellar primordium [72].

Ebf2 and PC Subtype Specification

Thus far we have treated PC development as though all PCs are the same. This is far from the case – indeed in the adult mouse cerebellum, multiple PC subtypes have been identified (e.g., zebrin II/aldolase C [7]; PLCβ3/4 [73]; HSP25 [74]: reviewed in [6]). The embryological origins of PC heterogeneity and pattern formation are only slowly coming into focus. PC subtype phenotype is cerebellum intrinsic and independent of neural activity (e.g., [75]) or afferent innervation [76, 77]. Cerebellar compartmentation appears to start at ~E10 in the VZ of the fourth ventricle but likely not sooner (e.g., [78–81]). The first stage likely occurs when PCs undergo terminal mitosis between E10 and E13 [58] in the *Ptf1a*-expressing progenitor domain of the VZ [43, 69]. Birthdating studies have identified two distinct PC populations: an early-born cohort (E10–E11.5) fated to become zebrin II^{+} and a late-born cohort (E11.5–E13) fated to become zebrin II^{-} [82, 83]. However, individual PC stripes do not have a clonal origin [80]. There is also a direct correlation between PC birthdates and their adult stripe location, suggesting that both subtype specification and positional information (i.e., which zone or stripe the PC will occupy) may be acquired at this time (e.g., [82, 84–86]).

Several regulatory genes are implicated in PC progenitor development. Among them, *Early B-cell factor 2* (*Ebf2*) [87] belongs to a family of atypical basic helix-loop-helix transcription factors that do not possess a basic domain and instead

feature a unique DNA-binding domain. This family includes three transcriptional activators (EBF1–3) and one repressor (EBF4) (reviewed in [88, 89]). *Ebf2* is expressed in a subset of late-born PC progenitors fated to populate zebrin II$^-$ parasagittal stripes, and in *Ebf2* null mutants the cerebellum features a selective loss of zebrin II$^-$ PCs.

Upon cell cycle exit, late-born PC progenitors start expressing *Ebf2* and migrate toward the PC plate. Posterior-born PCs migrate tangentially at first and then follow radial glial fibers, projecting their axons ventrally into the prospective white matter [90]. Conversely, anteriorly born PCs migrate radially into the PC plate, also following radial glial fibers, to populate anterior regions of the cerebellar cortex. Migration of this latter population is reelin (RELN) dependent and selectively delayed in *Ebf2* null PCs, which accumulate before birth as an ectopic layer just above the VZ in the anterior third of the cerebellar anlage. A significant fraction of these PCs, many of which express neurogranin [91], dies by apoptosis [92, 93]. *Ebf2* is required to support survival of late-born PCs at birth and accomplishes this by transactivating the *insulin-like growth factor* (*Igf1*) gene. In postnatal *Ebf2* null cerebella, *Igf1* expression is downregulated, with a resulting impairment of IGF-1 signal transduction [93]. Finally, some of the *Ebf2* null PCs that survive lose their PC subtype specification features and trans-differentiate into zebrin II$^+$ PCs – the only genetic manipulation thus far shown to subvert PC subtype specification [92]. In fact, *Ebf2* acts to repress the zebrin II$^+$ phenotype in late-born PCs [85]. Further studies, employing conditional mutants, are required to determine at which stage of postmitotic PC precursor development EBF2 acts to specify PC subtype. The results of genetic fate mapping experiments (GGC et al.: unpublished data) suggest that *Ebf2* is expressed transiently in all PC progenitors, only to be restricted to late-born ones by the end of embryogenesis. The pathways that lead to further subtype specification (e.g., the HSP25$^{+/-}$ distinction within the zebrin II$^+$ family: [74]) have not yet been explored.

Embryonic PC Cluster Formation

Newborn PCs migrate dorsally into the cerebellar anlage where they aggregate by ~E17 into a reproducible array of clusters that already contains multiple distinct molecular PC phenotypes ([6], reviewed in [83], e.g., Fig. 2 [94–97]). These clusters are the targets by which cerebellar afferents and many interneurons become topographically ordered (reviewed in [5, 28]).

The mechanism that converts the PC plate into the elaborate array of embryonic PC clusters – >50 are recorded [97, 98] – is not well understood. As PCs migrate toward the cerebellar surface, the early-born (E10–E11.5: *Ebf2*$^-$; future zebrin II$^+$) PC lamina interdigitates with the more superficial late-born (E11.5–E13; *Ebf2*$^+$; future zebrin II$^-$) layer with the result that the stereotyped array of clusters emerges. Whether this migration is the mechanism that specifies cluster architecture or whether the clusters are already specified in the cerebellar plate (or are even preformed in the

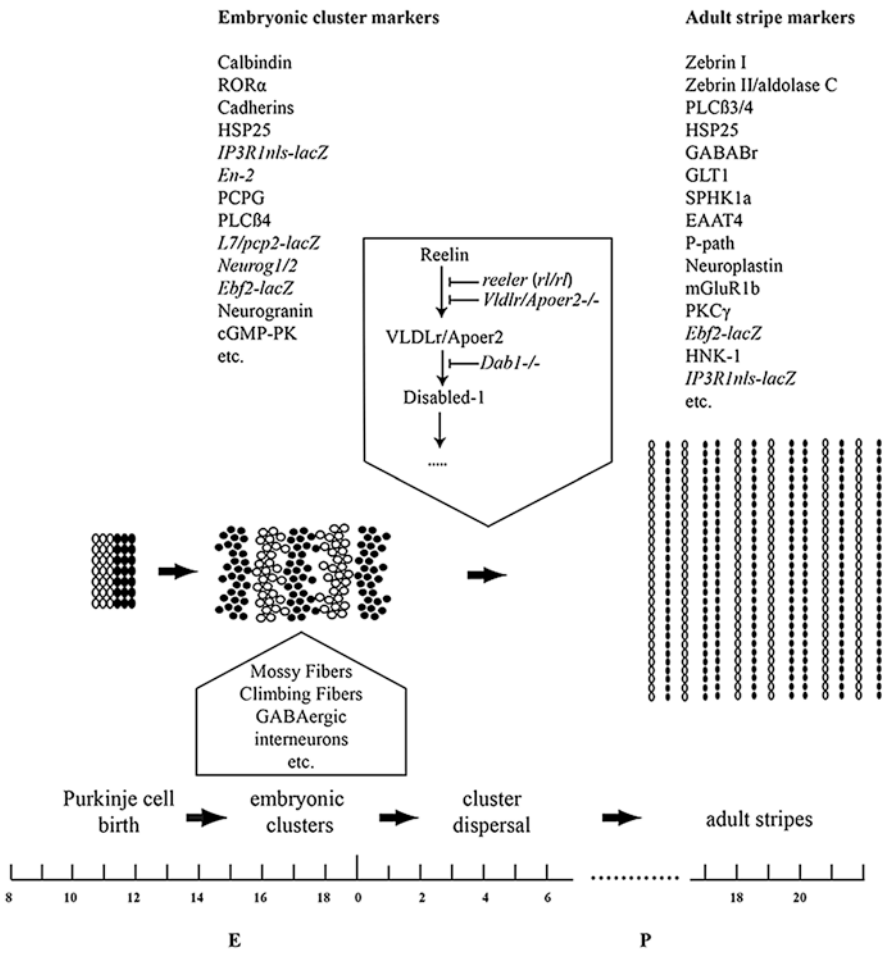

Fig. 2 From clusters to stripes. Embryonic clusters condense by migration from the cerebellar plate between E14 and E18 (mouse). At this stage numerous expression markers reveal that the PC population is already heterogeneous (exactly how many distinct phenotypes are present is not known, in part because of the paucity of double-labeling studies). The embryonic PC clusters also serve as a staging area to amass, organize, and restrict cerebellar afferents and interneurons. Starting perinatally signals via the RELN-Dab1 pathway trigger cluster dispersal into the adult cluster array by about P20. As for the embryonic clusters, the exact number of stripe phenotypes is not certain – at least ten may be identified based on expression data and mutant phenotypes. References to the lists of embryonic and adult cerebellar compartment markers may be found in [6]

VZ, i.e., some version of the protomap model proposed by Rakic for the neocortex: reviewed in [99]) is not known. The cellular processes that guide cluster formation are not understood, but grafts of dissociated PCs also organize into discrete, ectopic zebrin II^{+}/zebrin II^{-} aggregates [100], pointing to cell-cell adhesion molecules as possible organizers: cadherins (reviewed in [101]) and integrins (e.g., [102]) are

possible candidates. Also during this same period, the cerebellar anlage undergoes a 90° rotation, which converts the embryonic rostrocaudal axis into the mediolateral axis of the cerebellar primordium [81], so perhaps the adult stripe array ultimately derives from the anteroposterior patterning of dorsal rhombomere 1.

From Clusters to Zones and Stripes

Boundaries running from medial to lateral divide the cerebellar cortex into transverse zones. By combining different sources of evidence – molecular, genetic, and hodological – four highly conserved transverse boundaries, and hence five transverse zones, have been delineated in the adult mouse vermis (e.g., [103–105]): the anterior zone (AZ: ~ lobules I–V: reviewed in [104]), central zone anterior (CZa: ~lobule VI) and posterior (CZp), the posterior zone (PZ: ~lobules VIII to dorsal IX: reviewed in [105]), and the nodular zone (NZ). Each transverse zone is then further subdivided into a reproducible array of parasagittal stripes (e.g., revealed by using zebrin II/aldolase C [7, 8]: for zebrin II$^{+/-}$ stripes, these are labeled P^{+} and P^{-}, e.g., zebrin II [106], phospholipase (PL) Cß3/Cß4 [73]), the small heat shock protein HSP25 [74], or *L7/pcp2-lacZ* transgene expression (reviewed in [6, 103]).

PC stripes are discontinuous across transverse boundaries so it seems plausible that the zones precede stripes in development, but whether transverse zones form prior to the PC clusters or at the same time is speculative. Transverse boundaries are certainly present in the embryonic cerebellum. The AZ/CZa boundary between lobules V and VI can be identified both in neonates and adults by the expression domains of numerous molecules (e.g., calbindin [103], reviewed in [107]) and is a developmental phenotype restriction boundary for several cerebellar mutations. (In some cases, the mutant phenotype is associated with defects in the AZ (e.g., [108]), lurcher Grid2Lc-J: [109], and *cerebellar-deficient folia* (Ctnna2cdf: [110]); in others – for example, the BETA2/*NeuroD1* null [111] – it is the posterior cerebellar zones that are the most affected.) Finally, a granular layer lineage restriction boundary also lies in the anterior face of lobule VI, indicating that granule cells either side of the boundary derive from different lineages [112]. The CZa/CZp boundary [113] is a perinatal restriction boundary for FoxP2 [107], *Gli* [95], and HNK-1 expression [114]. The CZp/PZ boundary that separates lobule VII from lobule VIII is revealed in the perinatal cerebellum by FoxP2 [98, 107], PLCβ4 [115] and HSP25 [116] expression which is associated with a phenotypic abnormality in the *lurcher* (*Grid*^{Lc}) mouse [109]. Finally, the most caudal transverse boundary in the adult mouse (PZ/NZ) lies near the base of the posterolateral fissure between lobules IX and X. A transverse boundary has also been located in the same area during development as a restriction boundary for the expression of En2 [95] and FoxP2 [96]. A granular layer transverse boundary in embryonic stem cell chimeras is also located at around the PZ/NZ boundary [112].

Starting at around E18, the embryonic clusters transform into adult stripes triggered by RELN signaling (reviewed in [117, 118]). Because PC dispersal and the

associated development of cerebellar foliation occur almost entirely along the rostrocaudal axis, each cluster becomes stretched out into a long, narrow stripe.

RELN is secreted by both the external granular layer and glutamatergic projection neurons of the cerebellar nuclei [117] and binds to two PC receptors – the apolipoprotein E receptor 2 (Apoer2) and the very low-density lipoprotein receptor (VLDLR: [119, 120]). Both receptors are required for normal stripe formation, and if RELN is absent (e.g., the *reeler* mouse (*Reln*^{rl})), PC cluster dispersal is blocked, and the adult mouse retains the embryonic cluster morphology and is ataxic (reviewed in [24]). RELN binding induces Apoer2/Vldlr receptor clustering [121], which triggers a protein kinase cascade and tyrosine phosphorylation of the docking protein Disabled1 (DAB: [122–126]) by Fyn and Src [127, 128], leading eventually to a drop in mutual PC-PC adhesion, possibly via integrins. In parallel, DAB1 phosphorylation also activates Rac and Rho GTPases, which control actin filament assembly [129]. Together cytoskeletal and cell adhesion changes are thought to permit the embryonic PC clusters to disperse into stripes. That being said, it is not clear whether cluster dispersal requires the active migration of PCs or is the passive consequence of lobule formation.

However, the RELN pathway is not that straightforward. First, while expression mapping suggests that all PCs express both Apoer2 and Vldlr RELN receptors, mutations in individual receptors (*Apoer2*$^{-/-}$ and *Vldlr*$^{-/-}$ nulls; *Apoer2*$^{+/-}$:Vldlr$^{+/-}$ double heterozygotes) result in specific partial *reeler* phenotypes with some clusters dispersing normally, while others remain ectopic ([130]; divergent roles are also seen in the developing cerebral cortex [131]). Similar behavior is seen in several naturally occurring mutants. For example, *meander tail* (mea2J [132]), *rostral cerebellar malformation* (Unc5crcm [108]), 1998), and *cerebellar-deficient folia* (Ctnna2cdf [110]) all display selective PC ectopias that are restricted to the zebrin II^{-} phenotype (and because zebrin II^{-} PCs are preferentially located in the AZ, it is the anterior vermis that is most severely affected). In a more complex model – the *weaver* (*Kcnj6*^{wv}) mouse – PC cluster dispersal failure is restricted to zebrin II^{+}/HSP25^{+} stripes in the CZa/CZp [133]. The GIRK2 protein mutated in *weaver* [134] is expressed by all PCs so the molecular basis of the selective PC ectopias is unknown.

The relationship between the embryonic cluster topography and the zone and stripe pattern of the adult is not fully mapped. Because a few markers are expressed consistently in both clusters and stripes (FOXP2: [98], several are, for example, PLCß4 [115], an IP3R1 promoter-nls-lacZ transgene [135]), but others are only expressed in stripes at one stage or show very different expression patterns perinatally versus the adult, e.g., HSP25 (e.g., HSP25 [116], lysosomal acid phosphatase 2 [136]), there is limited evidence of the continuity of the cerebellar topographic map from perinate to adult. In theory, three relationships might occur: one embryonic cluster might form a single adult stripe; one cluster might split to yield more than one stripe; or several clusters might combine into a single stripe (Fig. 3). In fact, all three possibilities have been described. In several cases, the one cluster = one stripe model seems very likely (e.g., [83, 98, 135]). However, other examples are more complex. For example, the so-called P1^{-} stripe in the AZ vermis clearly derives from three distinct embryonic clusters, which abut (as revealed by using

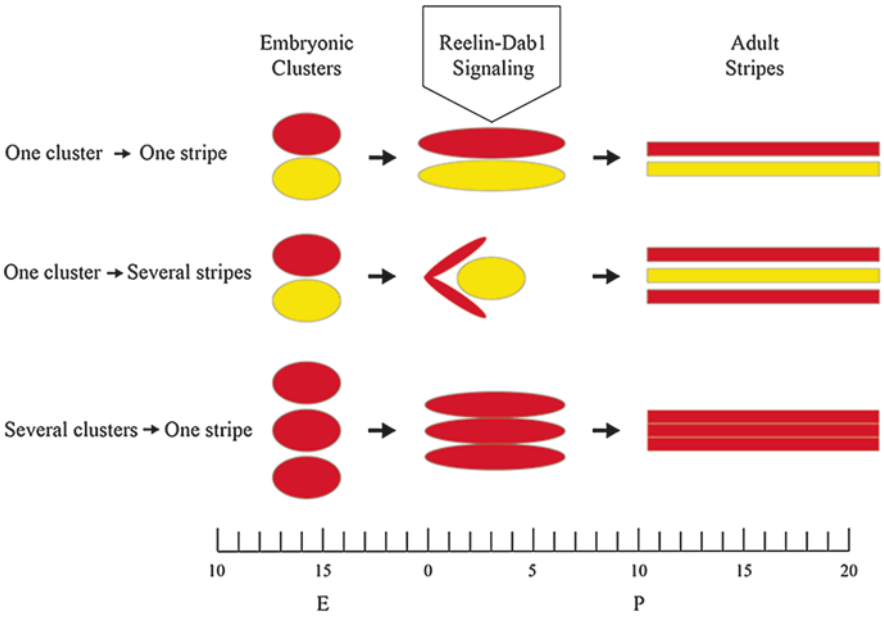

Fig. 3 Three models for the transformation of embryonic PC clusters into adult stripes: one embryonic cluster forms one adult stripe; one cluster splits to yield several stripes; or several clusters combine into a single stripe. All three models are found

PLCß4 expression [115]). An alternative – and perhaps better – description is that the apparently homogeneous P1$^-$ stripe in the adult (all zebrin II$^-$/PLCß4$^+$) actually comprises three distinct sub-stripes. The triplet structure is also seen in the afferent mossy fiber projections (where cuneocerebellar and spinocerebellar pathways terminate in different sub-stripes: [137, 138]) and in the expression of an *L7/pcp2-lacZ* transgene [103]. A similar covert heterogeneity is seen in ostensibly homogeneous zebrin II$^+$ stripes when co-labeled for HSP25 [74]. Last, single clusters may give rise to multiple stripes. For example, inducible fate mapping with a *Pcp2-CreER-IRES-hAP* transgene showed three cluster pairs contribute to nine adult stripes [28].

Finally, a striking feature of adult cerebellar topography is the high reproducibility between individuals and the concomitant low error rate (e.g., zebrin II$^+$ PCs are very rarely seen in zebrin II$^-$ stripes). If stripes derive from clusters, and stripes have no errors, then either clusters have no errors (and migration from the VZ to the clusters is perfect) or errors that occur during cluster formation and dispersal are selectively eliminated. In this context it is interesting that many PCs – perhaps as many as a third – undergo cell death by apoptosis during the perinatal period [139]. This suggests the hypothesis that perinatal apoptosis eliminates those PCs that wind up in the wrong embryonic cluster. PC ectopia is not lethal per se: for example, PCs located ectopically may survive indefinitely. Rather, the hypothesis evokes a community effect, such that being in the wrong cluster leads to apoptosis. In support of the idea that apoptosis

refines topography, studies of naturally occurring cell death in the cerebellum identified hot spots of PC apoptosis that correlate with stripe boundaries in the adult [140]. However, preliminary experiments do not support the hypothesis. Deleting the Bcl-2-/BH3-associated apoptotic protein BAX inhibits perinatal PC death (BAX is expressed in PCs perinatally [141] and *Bax−/−* mice have a 30% excess of PCs over controls (e.g., [142]). Nevertheless, the frequency of targeting errors was unaffected (RH and Y. Wang: unpublished data). Therefore, the remarkable reproducibility of the cerebellar map does not seem to result from perinatal error correction.

Conclusions

Early stages of PC development affect both susceptibility and outcome of several motor and cognitive disorders. Cerebellar development is protracted (from E7 to P30) and complex (at least two germinal zones, multiple migration pathways, etc.) so it is unsurprising that it represents a large target for developmental disruption. Spinocerebellar ataxia type 1 provides an example of this: transgenic mice in which expression of the expanded *ATXN1* transgene is delayed until after the cerebellum has matured display a reduced disease phenotype, suggesting that mutant *ATXN1* interacts with a pathway involved in PC development, likely by affecting *RORa* expression. Thus, compromising PC development appears to contribute to the severity of neurodegeneration [143]. Equally striking, recent evidence has linked PC development to the pathogenesis of autistic spectrum disorders (reviewed in [144]). In particular, selective deletion of the *Tsc1* gene in the PC lineage from conditional knockout mice has been found to cause a decrease in PC number, increased spine density, and autistic-like alterations of social behavior [145]. One of many insults thought to trigger autism is maternal fever [146]. Possibly related to the putative role of the cerebellum in autism, we recently found that immune activation and fever in pregnant mice between E13 and E15 resulted in adult progeny with significantly wider zebrin II$^{+/-}$ stripes, greater numbers of PCs, poorer motor performance, and impaired social interactions in adolescence [147].

Finally, what are the prospects that early intervention might afford therapeutic advantages? While fast progress has been made in recent years, plenty remains to be learned in regard to the signals that instruct VZ progenitors to adopt PC versus GABAergic interneuron fate. To our knowledge, protocols aimed at producing PCs from ES/iPS cells in vitro are based on selection of early PC progenitors that express lineage-specific surface markers [148]. The identification of additional factors cooperating with PTF1a and OLIG2 in specifying the earliest PC progenitors should improve the efficiency of those protocols and make it possible to generate autologous PCs from iPS cells or via direct reprogramming. These short-range projection neurons produced in vitro may eventually constitute a source of cell replacement in patients affected by certain types of degenerative ataxias.

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References

1. Hawkes R, Gravel C. The modular cerebellum. Prog Neurobiol. 1991;36(4):309–27. PubMed PMID: 1871318.
2. Hawkes R. An anatomical model of cerebellar modules. Prog Brain Res. 1997;114:39–52. PubMed PMID: 9193137.
3. Eisenman LM. Antero-posterior boundaries and compartments in the cerebellum: evidence from selected neurological mutants. Prog Brain Res. 2000;124:23–30. PubMed PMID: 10943114.
4. Sillitoe R, Joyner A. Morphology, molecular codes, and circuitry produce the three-dimensional complexity of the cerebellum. Annu Rev Cell Dev Biol. 2007;23:549–77. Epub ahead of print.
5. Apps R, Hawkes R. Cerebellar cortical organization: a one-map hypothesis. Nat Rev Neurosci. 2009;10(9):670–81. PubMed PMID: 19693030. Epub 2009/08/21. eng.
6. Armstrong CL, Hawkes R. Pattern formation in the cerebellum. San Rafael: Morgan and Claypool; 2013.
7. Brochu G, Maler L, Hawkes R. Zebrin II: a polypeptide antigen expressed selectively by Purkinje cells reveals compartments in rat and fish cerebellum. J Comp Neurol. 1990;291(4):538–52. PubMed PMID: 2329190.
8. Ahn AH, Dziennis S, Hawkes R, Herrup K. The cloning of zebrin II reveals its identity with aldolase C. Development. 1994;120(8):2081–90. PubMed PMID: 7925012.
9. Voogd J, Ruigrok TJ. The organization of the corticonuclear and olivocerebellar climbing fiber projections to the rat cerebellar vermis: the congruence of projection zones and the zebrin pattern. J Neurocytol. 2004;33(1):5–21. PubMed PMID: 15173629.
10. Sillitoe RV, Chung SH, Fritschy JM, Hoy M, Hawkes R. Golgi cell dendrites are restricted by Purkinje cell stripe boundaries in the adult mouse cerebellar cortex. J Neurosci. 2008;28(11):2820–6. PubMed PMID: 18337412.
11. Consalez GG, Hawkes R. The compartmental restriction of cerebellar interneurons. Front Neural Circ. 2012;6:123. PubMed PMID: 23346049. Pubmed Central PMCID: 3551280. Epub 2013/01/25.
12. Scott TG. A unique pattern of localization within the cerebellum. Nature. 1963;200:793. PubMed PMID: 14087025.
13. Ramó y Cajal S. Histologie du Système Nerveux de l'Homme et des Vertébrés. 1911.
14. Hatten ME, Heintz N. Mechanisms of neural patterning and specification in the developing cerebellum. Ann Rev Neurosci. 1995;18:385–408.
15. Altman J, Bayer SA. Development of the cerebellar system in relation to its evolution, structure, and functions. Boca Raton: CRC Press; 1997.
16. Sotelo C. Cellular and genetic regulation of the development of the cerebellar system. Prog Neurobiol. 2004;72(5):295–339. PubMed PMID: 15157725.
17. Carletti B, Rossi F. Neurogenesis in the cerebellum. Neuroscientist. 2008;14(1):91–100. PubMed PMID: 17911211. Epub 2007/10/04. eng.
18. Hoshino M. Neuronal subtype specification in the cerebellum and dorsal hindbrain. Develop Growth Differ. 2012;54(3):317–26. PubMed PMID: 22404503.
19. Leto K, Arancillo M, Becker EB, Buffo A, Chiang C, Ding B, et al. Consensus paper: cerebellar develpoment. Cerebellum. 2015;15:789–828. PubMed PMID: 26439486. Pubmed Central PMCID: PMC4846577.

20. Smeyne RJ, Chu T, Lewin A, Bian F, Sanlioglu SC, Kunsch C, et al. Local control of granule cell generation by cerebellar Purkinje cells. Mol Cell Neurosci. 1995;6(3):230–51.
21. Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron. 1999;22(1):103–14. PubMed PMID: 10027293.
22. Dahmane N, Ruiz-i-Altaba A. Sonic hedgehog regulates the growth and patterning of the cerebellum. Development. 1999;126(14):3089–100.
23. Wallace VA. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. Curr Biol. 1999;9:445–8.
24. Goffinet AM. The embryonic development of the cerebellum in normal and reeler mutant mice. Anat Embryol (Berl). 1983;168(1):73–86. PubMed PMID: 6650858.
25. Paradies MA, Eisenman LM. Evidence of early topographic organization in the embryonic olivocerebellar projection: a model system for the study of pattern formation processes in the central nervous system. Dev Dyn. 1993;197(2):125–45. PubMed PMID: 8219355.
26. Grishkat HL, Eisenman LM. Development of the spinocerebellar projection in the prenatal mouse. J Comp Neurol. 1995;363(1):93–108. PubMed PMID: 8682940.
27. White JJ, Sillitoe RV. Postnatal development of cerebellar zones revealed by neurofilament heavy chain protein expression. Front Neuroanat. 2013;7:9. PubMed PMID: 23675325. Pubmed Central PMCID: PMC3648691.
28. Sillitoe RV, Gopal N, Joyner AL. Embryonic origins of ZebrinII parasagittal stripes and establishment of topographic Purkinje cell projections. Neuroscience. 2009;162(3):574–88. PubMed PMID: 19150487. Pubmed Central PMCID: 2716412. Epub 2009/01/20. eng.
29. Blatt GJ, Eisenman LM. Topographic and zonal organization of the olivocerebellar projection in the reeler mutant mouse. J Comp Neurol. 1988;267(4):603–15. PubMed PMID: 2831252.
30. Reeber SL, Loeschel CA, Franklin A, Sillitoe RV. Establishment of topographic circuit zones in the cerebellum of scrambler mutant mice. Front Neural Circ. 2013;7:122. PubMed PMID: 23885237. Pubmed Central PMCID: PMC3717479.
31. Sillitoe RV, Vogel MW, Joyner AL. Engrailed homeobox genes regulate establishment of the cerebellar afferent circuit map. J Neurosci. 2010;30(30):10015–24. PubMed PMID: 20668186. Pubmed Central PMCID: PMC2921890.
32. Hallonet ME, Teillet MA, Le Douarin NM. A new approach to the development of the cerebellum provided by the quail-chick marker system. Development. 1990;108(1):19–31. PubMed PMID: 2351063. Epub 1990/01/01. eng.
33. Hallonet ME, Le Douarin NM. Tracing neuroepithelial cells of the mesencephalic and metencephalic alar plates during cerebellar ontogeny in quail-chick chimaeras. Eur J Neurosci. 1993;5(9):1145–55.
34. Hallonet M, Alvarado-Mallart RM. The chick/quail chimeric system: a model for early cerebellar development. Perspect Dev Neurobiol. 1997;5(1):17–31. PubMed PMID: 9509515.
35. Broccoli V, Boncinelli E, Wurst W. The caudal limit of Otx2 expression positions the isthmic organizer. Nature. 1999;9(401(6749)):164–8.
36. Li JY, Lao Z, Joyner AL. New regulatory interactions and cellular responses in the isthmic organizer region revealed by altering Gbx2 expression. Development. 2005;132(8):1971–81. PubMed PMID: 15790971.
37. Martinez S, Wassef M, Alvarado-Mallart RM. Induction of a mesencephalic phenotype in the 2-day-old chick prosencephalon is preceded by the early expression of the homeobox gene en. Neuron. 1991;6(6):971–81. PubMed PMID: 1675863.
38. Martinez S, Crossley PH, Cobos I, Rubenstein JL, Martin GR. FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on Otx2 expression. Development. 1999;126(6):1189–200. PubMed PMID: 10021338.
39. Martinez S, Alvarado-Mallart RM. Rostral cerebellum originates from the caudal portion of the so-called 'Mesencephalic' vesicle: a study using chick/quail chimeras. Eur J Neurosci. 1989;1(6):549–60. PubMed PMID: 12106114. Epub 1989/01/01. Eng.
40. Alvarez Otero R, Sotelo C, Alvarado-Mallart RM. Chick/quail chimeras with partial cerebellar grafts: an analysis of the origin and migration of cerebellar cells. J Comp Neurol. 1993;333(4):597–615. PubMed PMID: 7690372. Epub 1993/07/22. eng.

41. Marin F, Puelles L. Patterning of the embryonic avian midbrain after experimental inversions: a polarizing activity from the isthmus. Dev Biol. 1994;163:19–37.
42. Hidalgo-Sanchez M, Millet S, Bloch-Gallego E, Alvarado-Mallart RM. Specification of the meso-isthmo-cerebellar region: the Otx2/Gbx2 boundary. Brain Res Brain Res Rev. 2005;49(2):134–49. PubMed PMID: 16111544.
43. Hoshino M, Nakamura S, Mori K, Kawauchi T, Terao M, Nishimura YV, et al. Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron. 2005;47(2):201–13. PubMed PMID: 16039563.
44. Akazawa C, Ishibashi M, Shimizu C, Nakanishi S, Kageyama R. A mammalian helix-loop-helix factor structurally related to the product of Drosophila proneural gene atonal is a positive transcriptional regulator expressed in the developing nervous system. J Biol Chem. 1995;270(15):8730–8.
45. Anthony TE, Klein C, Fishell G, Heintz N. Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron. 2004;41(6):881–90. PubMed PMID: 15046721.
46. Seto Y, Nakatani T, Masuyama N, Taya S, Kumai M, Minaki Y, et al. Temporal identity transition from Purkinje cell progenitors to GABAergic interneuron progenitors in the cerebellum. Nat Commun. 2014;5:3337. PubMed PMID: 24535035.
47. Yamada M, Seto Y, Taya S, Owa T, Inoue YU, Inoue T, et al. Specification of spatial identities of cerebellar neuron progenitors by ptf1a and atoh1 for proper production of GABAergic and glutamatergic neurons. J Neurosci. 2014;34(14):4786–800. PubMed PMID: 24695699.
48. Sellick GS, Barker KT, Stolte-Dijkstra I, Fleischmann C, Coleman RJ, Garrett C, et al. Mutations in PTF1A cause pancreatic and cerebellar agenesis. Nat Genet. 2004;36(12):1301–5. PubMed PMID: 15543146.
49. Alder J, Cho NK, Hatten ME. Embryonic precursor cells from the rhombic lip are specified to a cerebellar granule neuron identity. Neuron. 1996;17(3):389–99. PubMed PMID: 8816703.
50. Wingate RJ. The rhombic lip and early cerebellar development. Curr Opin Neurobiol. 2001;11(1):82–8. PubMed PMID: 11179876. Epub 2001/02/17. eng.
51. Machold R, Fishell G. Math1 is expressed in temporally discrete pools of cerebellar rhombic-lip neural progenitors. Neuron. 2005;48(1):17–24. PubMed PMID: 16202705.
52. Wang VY, Rose MF, Zoghbi HY. Math1 expression redefines the rhombic lip derivatives and reveals novel lineages within the brainstem and cerebellum. Neuron. 2005;48(1):31–43. PubMed PMID: 16202707.
53. Fink AJ, Englund C, Daza RA, Pham D, Lau C, Nivison M, et al. Development of the deep cerebellar nuclei: transcription factors and cell migration from the rhombic lip. J Neurosci. 2006;26(11):3066–76. PubMed PMID: 16540585.
54. Englund C, Kowalczyk T, Daza RA, Dagan A, Lau C, Rose MF, et al. Unipolar brush cells of the cerebellum are produced in the rhombic lip and migrate through developing white matter. J Neurosci. 2006;26(36):9184–95. PubMed PMID: 16957075
55. Vong KI, Leung CK, Behringer RR, Kwan KM. Sox9 is critical for suppression of neurogenesis but not initiation of gliogenesis in the cerebellum. Mol Brain. 2015;8:25. PubMed PMID: 25888505. Pubmed Central PMCID: PMC4406026.
56. Machold RP, Kittell DJ, Fishell GJ. Antagonism between Notch and bone morphogenetic protein receptor signaling regulates neurogenesis in the cerebellar rhombic lip. Neural Develop. 2007;2:5. PubMed PMID: 17319963.
57. Masserdotti G, Badaloni A, Green YS, Croci L, Barili V, Bergamini G, et al. ZFP423 coordinates Notch and bone morphogenetic protein signaling, selectively up-regulating Hes5 gene expression. J Biol Chem. 2010;285(40):30814–24. PubMed PMID: 20547764. Pubmed Central PMCID: 2945575.
58. Miale IL, Sidman RL. An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp Neurol. 1961;4:277–96. PubMed PMID: 14473282.
59. Sekerkova G, Ilijic E, Mugnaini E. Time of origin of unipolar brush cells in the rat cerebellum as observed by prenatal bromodeoxyuridine labeling. Neuroscience. 2004;127(4):845–58. PubMed PMID: 15312897.

60. Kim EJ, Battiste J, Nakagawa Y, Johnson JE. Ascl1 (Mash1) lineage cells contribute to discrete cell populations in CNS architecture. Mol Cell Neurosci. 2008;38(4):595–606. PubMed PMID: 18585058. Epub 2008/07/01. eng.
61. Chizhikov VV, Lindgren AG, Currle DS, Rose MF, Monuki ES, Millen KJ. The roof plate regulates cerebellar cell-type specification and proliferation. Development. 2006;133(15):2793–804. PubMed PMID: 16790481.
62. Leto K, Carletti B, Williams IM, Magrassi L, Rossi F. Different types of cerebellar GABAergic interneurons originate from a common pool of multipotent progenitor cells. J Neurosci: Off J Soc Neurosci. 2006;26(45):11682–94. PubMed PMID: 17093090. Epub 2006/11/10. eng.
63. Leto K, Rossi F. Specification and differentiation of cerebellar GABAergic neurons. Cerebellum. 2012;11(2):434–5. PubMed PMID: 22090364.
64. Ju J, Liu Q, Zhang Y, Liu Y, Jiang M, Zhang L, et al. Olig2 regulates Purkinje cell generation in the early developing mouse cerebellum. Sci Rep. 2016;6:30711. PubMed PMID: 27469598. Pubmed Central PMCID: PMC4965836.
65. Mizuhara E, Minaki Y, Nakatani T, Kumai M, Inoue T, Muguruma K, et al. Purkinje cells originate from cerebellar ventricular zone progenitors positive for Neph3 and E-cadherin. Dev Biol. 2009. PubMed PMID: 20004188. Epub 2009/12/17. Eng.
66. Minaki Y, Nakatani T, Mizuhara E, Inoue T, Ono Y. Identification of a novel transcriptional corepressor, Corl2, as a cerebellar Purkinje cell-selective marker. Gene Expr Patterns. 2008;8(6):418–23. PubMed PMID: 18522874. Epub 2008/06/05. eng.
67. Mizuhara E, Nakatani T, Minaki Y, Sakamoto Y, Ono Y. Corl1, a novel neuronal lineage-specific transcriptional corepressor for the homeodomain transcription factor Lbx1. J Biol Chem. 2005;280(5):3645–55. PubMed PMID: 15528197.
68. Morales D, Hatten ME. Molecular markers of neuronal progenitors in the embryonic cerebellar anlage. J Neurosci. 2006;26:12226–36.
69. Zordan P, Croci L, Hawkes R, Consalez GG. Comparative analysis of proneural gene expression in the embryonic cerebellum. Dev Dyn. 2008;237(6):1726–35. PubMed PMID: 18498101. Epub 2008/05/24. eng.
70. Kim EJ, Hori K, Wyckoff A, Dickel LK, Koundakjian EJ, Goodrich LV, et al. Spatiotemporal fate map of neurogenin1 (Neurog1) lineages in the mouse central nervous system. J Comp Neurol. 2011;519(7):1355–70. PubMed PMID: 21452201. Epub 2011/04/01. eng.
71. Lundell TG, Zhou Q, Doughty ML. Neurogenin1 expression in cell lineages of the cerebellar cortex in embryonic and postnatal mice. Dev Dyn. 2009;238(12):3310–25. PubMed PMID: 19924827. Epub 2009/11/20. eng.
72. Florio M, Leto K, Muzio L, Tinterri A, Badaloni A, Croci L, et al. Neurogenin 2 regulates progenitor cell-cycle progression and Purkinje cell dendritogenesis in cerebellar development. Development. 2012;139(13):2308–20. PubMed PMID: 22669821. Pubmed Central PMCID: 3367442. Epub 2012/06/07. eng.
73. Sarna JR, Marzban H, Watanabe M, Hawkes R. Complementary stripes of phospholipase Cbeta3 and Cbeta4 expression by Purkinje cell subsets in the mouse cerebellum. J Comp Neurol. 2006;496(3):303–13. PubMed PMID: 16566000.
74. Armstrong CL, Krueger-Naug AM, Currie RW, Hawkes R. Constitutive expression of the 25-kDa heat shock protein Hsp25 reveals novel parasagittal bands of Purkinje cells in the adult mouse cerebellar cortex. J Comp Neurol. 2000;416(3):383–97. PubMed PMID: 10602096.
75. Seil FJ, Johnson ML, Hawkes R. Molecular compartmentation expressed in cerebellar cultures in the absence of neuronal activity and neuron-glia interactions. J Comp Neurol. 1995;356(3):398–407. PubMed PMID: 7642801.
76. Leclerc N, Gravel C, Hawkes R. Development of parasagittal zonation in the rat cerebellar cortex: MabQ113 antigenic bands are created postnatally by the suppression of antigen expression in a subset of Purkinje cells. J Comp Neurol. 1988;273(3):399–420. PubMed PMID: 2463281.
77. Wassef M, Sotelo C, Thomasset M, Granholm AC, Leclerc N, Rafrafi J, et al. Expression of compartmentation antigen zebrin I in cerebellar transplants. J Comp Neurol. 1990;294(2):223–34. PubMed PMID: 2332530.

78. Baader SL, Vogel MW, Sanlioglu S, Zhang X, Oberdick J. Selective disruption of “late onset” sagittal banding patterns by ectopic expression of engrailed-2 in cerebellar Purkinje cells. J Neurosci. 1999;19(13):5370–9. PubMed PMID: 10377347.
79. Mathis L, Bonnerot C, Puelles L, Nicolas JF. Retrospective clonal analysis of the cerebellum using genetic laacZ/lacZ mouse mosaics. Development. 1997;124(20):4089–104. PubMed PMID: 9374405. Epub 1997/11/28. eng.
80. Hawkes R, Faulkner-Jones B, Tam P, Tan SS. Pattern formation in the cerebellum of murine embryonic stem cell chimeras. Eur J Neurosci. 1998;10(2):790–3. PubMed PMID: 9749745.
81. Sgaier SK, Millet S, Villanueva MP, Berenshteyn F, Song C, Joyner AL. Morphogenetic and cellular movements that shape the mouse cerebellum; insights from genetic fate mapping. Neuron. 2005;45(1):27–40. PubMed PMID: 15629700.
82. Hashimoto M, Mikoshiba K. Mediolateral compartmentalization of the cerebellum is determined on the “birth date” of Purkinje cells. J Neurosci. 2003;23(36):11342–51. PubMed PMID: 14672998.
83. Larouche M, Hawkes R. From clusters to stripes: the developmental origins of adult cerebellar compartmentation. Cerebellum. 2006;5(2):77–88. PubMed PMID: 16818382.
84. Karam SD, Burrows RC, Logan C, Koblar S, Pasquale EB, Bothwell M. Eph receptors and ephrins in the developing chick cerebellum: relationship to sagittal patterning and granule cell migration. J Neurosci. 2000;20(17):6488–500. PubMed PMID: 10964955.
85. Chung S-H, Marzban H, Croci L, Consalez G, Hawkes R. Purkinje cell subtype specification in the cerebellar cortex: Ebf2 acts to repress the Zebrin II-positive Purkinje cell phenotype. Neuroscience. 2008;153:721–32.
86. Namba K, Sugihara I, Hashimoto M. Close correlation between the birth date of Purkinje cells and the longitudinal compartmentalization of the mouse adult cerebellum. J Comp Neurol. 2011;519(13):2594–614. PubMed PMID: 21456012.
87. Malgaretti N, Pozzoli O, Bosetti A, Corradi A, Ciarmatori S, Panigada M, et al. Mmot1, a new helix-loop-helix transcription factor gene displaying a sharp expression boundary in the embryonic mouse brain. J Biol Chem. 1997;272(28):17632–9. PubMed PMID: 9211912. Epub 1997/07/11. eng
88. Dubois L, Vincent A. The COE – Collier/Olf1/EBF – transcription factors: structural conservation and diversity of developmental functions. Mech Dev. 2001;108(1–2):3–12.
89. Liberg D, Sigvardsson M, Akerblad P. The EBF/Olf/Collier family of transcription factors: regulators of differentiation in cells originating from all three embryonal germ layers. Mol Cell Biol. 2002;22(24):8389–97. PubMed PMID: 12446759. Pubmed Central PMCID: PMC139877.
90. Miyata T, Ono Y, Okamoto M, Masaoka M, Sakakibara A, Kawaguchi A, et al. Migration, early axonogenesis, and Reelin-dependent layer-forming behavior of early/posterior-born Purkinje cells in the developing mouse lateral cerebellum. Neural Dev. 2010;5:23. PubMed PMID: 20809939. Pubmed Central PMCID: 2942860. Epub 2010/09/03. eng.
91. Larouche M, Che P, Hawkes R. Neurogranin expression identifies a novel array of Purkinje cell parasagittal stripes during mouse cerebellar development. J Comp Neurol. 2006;494(2):215–27.
92. Croci L, Chung SH, Masserdotti G, Gianola S, Bizzoca A, Gennarini G, et al. A key role for the HLH transcription factor EBF2COE2,O/E-3 in Purkinje neuron migration and cerebellar cortical topography. Development. 2006;133(14):2719–29. PubMed PMID: 16774995.
93. Croci L, Barili V, Chia D, Massimino L, van Vugt R, Masserdotti G, et al. Local insulin-like growth factor I expression is essential for Purkinje neuron survival at birth. Cell Death Differ. 2011;18(1):48–59. PubMed PMID: 20596079. Pubmed Central PMCID: 3131878.
94. Wassef M, Zanetta JP, Brehier A, Sotelo C. Transient biochemical compartmentalization of Purkinje cells during early cerebellar development. Dev Biol. 1985;111(1):129–37. PubMed PMID: 2993082.
95. Millen KJ, Hui CC, Joyner AL. A role for En-2 and other murine homologues of Drosophila segment polarity genes in regulating positional information in the developing cerebellum. Development. 1995;121(12):3935–45. PubMed PMID: 8575294.

96. Dastjerdi FV, Consalez GG, Hawkes R. Pattern formation during development of the embryonic cerebellum. Front Neuroanat. 2012;6:10. PubMed PMID: 22493569. Pubmed Central PMCID: 3318227. Epub 2012/04/12. eng.
97. Sugihara I, Fujita H. Peri- and postnatal development of cerebellar compartments in the mouse. Cerebellum. 2013;12(3):325–7. PubMed PMID: 23335119.
98. Fujita H, Sugihara I. FoxP2 expression in the cerebellum and inferior olive: development of the transverse stripe-shaped expression pattern in the mouse cerebellar cortex. J Comp Neurol. 2012;520(3):656–77. PubMed PMID: 21935935
99. Dehay C, Kennedy H. Cell-cycle control and cortical development. Nat Rev Neurosci. 2007;8(6):438–50. PubMed PMID: 17514197.
100. Rouse RV, Sotelo C. Grafts of dissociated cerebellar cells containing Purkinje cell precursors organize into zebrin I defined compartments. Exp Brain Res. 1990;82(2):401–7. PubMed PMID: 1704849.
101. Redies C, Neudert F, Lin J. Cadherins in cerebellar development: translation of embryonic patterning into mature functional compartmentalization. Cerebellum. 2011;10(3):393–408. PubMed PMID: 20820976.
102. Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, et al. Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. Neuron. 2001;31(3):367–79. PubMed PMID: 11516395.
103. Ozol K, Hayden JM, Oberdick J, Hawkes R. Transverse zones in the vermis of the mouse cerebellum. J Comp Neurol. 1999;412(1):95–111. PubMed PMID: 10440712.
104. Sillitoe RV, Marzban H, Larouche M, Zahedi S, Affanni J, Hawkes R. Conservation of the architecture of the anterior lobe vermis of the cerebellum across mammalian species. Prog Brain Res. 2005;148:283–97. PubMed PMID: 15661197.
105. Marzban H, Hawkes R. On the architecture of the posterior zone of the cerebellum. Cerebellum. 2011;10(3):422–34. PubMed PMID: 20838950.
106. Sillitoe RV, Hawkes R. Whole-mount immunohistochemistry: a high-throughput screen for patterning defects in the mouse cerebellum. J Histochem Cytochem. 2002;50(2):235–44. PubMed PMID: 11799142.
107. Dastjerdi FV. Transverse boundaries in the embryonic cerebellar cortex of the mouse. Alberta: University of Calgary; 2012.
108. Eisenman LM, Brothers R. Rostral cerebellar malformation (rcm/rcm): a murine mutant to study regionalization of the cerebellum. J Comp Neurol. 1998;394(1):106–17. PubMed PMID: 9550145.
109. Tano D, Napieralski JA, Eisenman LM, Messer A, Plummer J, Hawkes R. Novel developmental boundary in the cerebellum revealed by zebrin expression in the lurcher (Lc/+) mutant mouse. J Comp Neurol. 1992;323(1):128–36. PubMed PMID: 1430312.
110. Beierbach E, Park C, Ackerman SL, Goldowitz D, Hawkes R. Abnormal dispersion of a Purkinje cell subset in the mouse mutant cerebellar deficient folia (cdf). J Comp Neurol. 2001;436(1):42–51. PubMed PMID: 11413545.
111. Cho JH, Tsai MJ. Preferential posterior cerebellum defect in BETA2/NeuroD1 knockout mice is the result of differential expression of BETA2/NeuroD1 along anterior-posterior axis. Dev Biol. 2006;290(1):125–38. PubMed PMID: 16368089.
112. Hawkes R, Beierbach E, Tan SS. Granule cell dispersion is restricted across transverse boundaries in mouse chimeras. Eur J Neurosci. 1999;11(11):3800–8. PubMed PMID: 10583469.
113. Marzban H, Kim CT, Doorn D, Chung SH, Hawkes R. A novel transverse expression domain in the mouse cerebellum revealed by a neurofilament-associated antigen. Neuroscience. 2008;153:721–32.
114. Marzban H, Sillitoe RV, Hoy M, Chung SH, Rafuse VF, Hawkes R. Abnormal HNK-1 expression in the cerebellum of an N-CAM null mouse. J Neurocytol. 2004;33(1):117–30. PubMed PMID: 15173636.
115. Marzban H, Chung S, Watanabe M, Hawkes R. Phospholipase Cbeta4 expression reveals the continuity of cerebellar topography through development. J Comp Neurol. 2007;502(5):857–71. PubMed PMID: 17436294.

116. Armstrong CL, Krueger-Naug AM, Currie RW, Hawkes R. Constitutive expression of heat shock protein HSP25 in the central nervous system of the developing and adult mouse. J Comp Neurol. 2001;434(3):262–74. PubMed PMID: 11331528.
117. D'Arcangelo G. Reelin in the years: controlling neuronal migration and maturation in the mammalian brain. Adv Neurosci. 2014;2014:4–19.
118. Bock HH, May P. Canonical and non-canonical Reelin signaling. Front Cell Neurosci. 2016;10:166. PubMed PMID: 27445693. Pubmed Central PMCID: PMC4928174.
119. Trommsdorff M, Gotthardt M, Hiesberger T, Shelton J, Stockinger W, Nimpf J, et al. Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell. 1999;97(6):689–701. PubMed PMID: 10380922.
120. Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, et al. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. Neuron. 1999;24(2):481–9. PubMed PMID: 10571241.
121. Strasser V, Fasching D, Hauser C, Mayer H, Bock HH, Hiesberger T, et al. Receptor clustering is involved in Reelin signaling. Mol Cell Biol. 2004;24(3):1378–86. PubMed PMID: 14729980. Pubmed Central PMCID: PMC321426.
122. Goldowitz D, Cushing RC, Laywell E, D'Arcangelo G, Sheldon M, Sweet HO, et al. Cerebellar disorganization characteristic of reeler in scrambler mutant mice despite presence of reelin. J Neurosci: Off J Soc Neurosci. 1997;17(22):8767–77. PubMed PMID: 9348346. Epub 1997/11/14. eng.
123. Howell BW, Hawkes R, Soriano P, Cooper JA. Neuronal position in the developing brain is regulated by mouse disabled-1. Nature. 1997;389(6652):733–7. PubMed PMID: 9338785.
124. Sheldon M, Rice DS, D'Arcangelo G, Yoneshima H, Nakajima K, Mikoshiba K, et al. Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. Nature. 1997;389(6652):730–3. PubMed PMID: 9338784.
125. Gallagher E, Howell BW, Soriano P, Cooper JA, Hawkes R. Cerebellar abnormalities in the disabled (mdab1-1) mouse. J Comp Neurol. 1998;402(2):238–51. PubMed PMID: 9845246.
126. Rice DS, Sheldon M, D'Arcangelo G, Nakajima K, Goldowitz D, Curran T. Disabled-1 acts downstream of Reelin in a signaling pathway that controls laminar organization in the mammalian brain. Development. 1998;125(18):3719–29. PubMed PMID: 9716537.
127. Howell BW, Gertler FB, Cooper JA. Mouse disabled (mDab1): a Src binding protein implicated in neuronal development. EMBO J. 1997;16(1):121–32. PubMed PMID: 9009273. Pubmed Central PMCID: PMC1169619.
128. Bock HH, Herz J. Reelin activates SRC family tyrosine kinases in neurons. Curr Biol. 2003;13(1):18–26. PubMed PMID: 12526740.
129. Chung CY, Funamoto S, Firtel RA. Signaling pathways controlling cell polarity and chemotaxis. Trends Biochem Sci. 2001;26(9):557–66. PubMed PMID: 11551793.
130. Larouche M, Beffert U, Herz J, Hawkes R. The Reelin receptors Apoer2 and Vldlr coordinate the patterning of Purkinje cell topography in the developing mouse cerebellum. PLoS One. 2008;3(2):e1653. PubMed PMID: 18301736. Pubmed Central PMCID: 2242849. Epub 2008/02/28. eng.
131. Hack I, Hellwig S, Junghans D, Brunne B, Bock HH, Zhao S, et al. Divergent roles of ApoER2 and Vldlr in the migration of cortical neurons. Development. 2007;134(21):3883–91. PubMed PMID: 17913789.
132. Ross ME, Fletcher C, Mason CA, Hatten ME, Heintz N. Meander tail reveals a discrete developmental unit in the mouse cerebellum. Proc Natl Acad Sci U S A. 1990;87(11):4189–92. PubMed PMID: 2349228. Pubmed Central PMCID: PMC54073.
133. Armstrong C, Hawkes R. Selective Purkinje cell ectopia in the cerebellum of the weaver mouse. J Comp Neurol. 2001;439(2):151–61. PubMed PMID: 11596045.
134. Slesinger PA, Patil N, Liao YJ, Jan YN, Jan LY, Cox DR. Functional effects of the mouse weaver mutation on G protein-gated inwardly rectifying K+ channels. Neuron. 1996;16(2):321–31. PubMed PMID: 8789947.

135. Furutama D, Morita N, Takano R, Sekine Y, Sadakata T, Shinoda Y, et al. Expression of the IP3R1 promoter-driven nls-lacZ transgene in Purkinje cell parasagittal arrays of developing mouse cerebellum. J Neurosci Res. 2010;88(13):2810–25. PubMed PMID: 20632399.
136. Bailey K, Rahimi Balaei M, Mehdizadeh M, Marzban H. Spatial and temporal expression of lysosomal acid phosphatase 2 (ACP2) reveals dynamic patterning of the mouse cerebellar cortex. Cerebellum. 2013;12(6):870–81. PubMed PMID: 23780826.
137. Akintunde A, Eisenman LM. External cuneocerebellar projection and Purkinje cell zebrin II bands: a direct comparison of parasagittal banding in the mouse cerebellum. J Chem Neuroanat. 1994;7(1–2):75–86. PubMed PMID: 7802972.
138. Ji Z, Hawkes R. Topography of Purkinje cell compartments and mossy fiber terminal fields in lobules II and III of the rat cerebellar cortex: spinocerebellar and cuneocerebellar projections. Neuroscience. 1994;61(4):935–54. PubMed PMID: 7530818.
139. Dusart I, Guenet JL, Sotelo C. Purkinje cell death: differences between developmental cell death and neurodegenerative death in mutant mice. Cerebellum. 2006;5(2):163–73. PubMed PMID: 16818391. Epub 2006/07/05. eng.
140. Jankowski J, Miething A, Schilling K, Baader SL. Physiological Purkinje cell death is spatiotemporally organized in the developing mouse cerebellum. Cerebellum. 2009;8(3):277–90. PubMed PMID: 19238501.
141. Gillardon F, Baurle J, Wickert H, Grusser-Cornehls U, Zimmermann M. Differential regulation of bcl-2, bax, c-fos, junB, and krox-24 expression in the cerebellum of Purkinje cell degeneration mutant mice. J Neurosci Res. 1995;41(5):708–15. PubMed PMID: 7563251.
142. Vogel MW. Cell death, Bcl-2, Bax, and the cerebellum. Cerebellum. 2002;1(4):277–87. PubMed PMID: 12879966
143. Serra HG, Duvick L, Zu T, Carlson K, Stevens S, Jorgensen N, et al. RORalpha-mediated Purkinje cell development determines disease severity in adult SCA1 mice. Cell. 2006;127(4):697–708. PubMed PMID: 17110330.
144. Basson MA, Wingate RJ. Congenital hypoplasia of the cerebellum: developmental causes and behavioral consequences. Front Neuroanat. 2013;7:29. PubMed PMID: 24027500. Pubmed Central PMCID: PMC3759752.
145. Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, et al. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. Nature. 2012;488(7413):647–51. PubMed PMID: 22763451. Epub 2012/07/06. eng.
146. Zerbo O, Iosif AM, Walker C, Ozonoff S, Hansen RL, Hertz-Picciotto I. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) study. J Autism Dev Disord. 2013;43(1):25–33. PubMed PMID: 22562209. Pubmed Central PMCID: PMC3484245.
147. Aavani T, Rana SA, Hawkes R, Pittman QJ. Maternal immune activation produces cerebellar hyperplasia and alterations in motor and social behaviors in male and female mice. Cerebellum. 2015;14(5):491–505. PubMed PMID: 25863812.
148. Muguruma K, Nishiyama A, Ono Y, Miyawaki H, Mizuhara E, Hori S, et al. Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. Nat Neurosci. 2010;13(10):1171–80. PubMed PMID: 20835252. Epub 2010/09/14. eng.