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Rautenlippe Redux – toward a unified view of the precerebellar rhombic lip

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Abstract

The rhombic lip (aka rautenlippe) is a germinative neuroepithelium rimming the opening of the hindbrain fourth ventricle during development. Studies spanning more than a century have shown that the rhombic lip produces numerous brainstem neuronal populations unique in their development and functions. While these studies have largely been anatomical in nature, recent applications of newer techniques such as genetic fate mapping and conditional mutagenesis have resolved the rhombic lip into numerous molecularly distinct progenitor domains along spatial and temporal axes that give rise to specific neuron subtypes and systems. This exciting convergence between anatomical and molecular definitions of the rhombic lip and its constituent progenitor populations provides now an important framework for further studies into the genetic basis of development and function of numerous hindbrain neuron types critical to life.

Neural systems comprising the mammalian brainstem are evolutionarily ancient and essential for life. Robust mechanisms must ensure their proper development during embryogenesis, yet such mechanisms have been challenging to define. In part, this is because of complexities particular to the developing hindbrain: flexures and extensive morphogenetic movements obscure delineation of the major neural axes over the course of development; constituent neurons organize into a complex patchwork of clusters called nuclei (by contrast to the more straightforward layered organization of many other brain regions); and finally, many neurons are highly migratory, residing ultimately in brainstem locations quite distant from their originating progenitor zones. No longer, though, do these complexities so impede studies of hindbrain development. Over the past decade, advances in fate mapping techniques along with sophisticated targeted mutations have made it possible to define quite precisely the primordium for, and molecular determinants of, many different brainstem neuron types. As one illustrative example, we review a series of studies on the brainstem precerebellar systems so critical for proper motor coordination.

There are two major precerebellar afferent systems: the mossy fiber (MF) system and the climbing fiber (CF) system. They are called “precerebellar” because they reside “before” the cerebellum with respect to anatomical position in the brainstem as well as circuit directionality. MF neurons cluster in multiple locations throughout the brainstem, with four prominent sites including the pontine gray nucleus (PGN), the reticulotegmental nucleus (RTN), the lateral reticular nucleus (LRN), and the external cuneate nucleus (ECN). These

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MF neurons provide excitatory input by extending axons to cerebellar granule cells. By contrast, CF neurons form a more localized yet extensive complex, the inferior olivary nuclei (ION) and extend excitatory connections to cerebellar Purkinje cells.

MF and CF neurons, despite their predominantly ventral and lateral locations in the brainstem, originate from neuroepithelia of the dorsal hindbrain. Thanks to molecular genetic advances of the past decade, we now even know the precise spatial, temporal and molecular coordinates of the originating progenitor pools, as well as specific gene products whose actions are critical for proper precerebellar fate specification. Further, we know from early work that the deployment of precerebellar neurons from their primordium is atypical. Rather than migrating radially along glia as individual neurons, newly born postmitotic precerebellar neurons migrate as cohorts streaming tangential to the radial axis. MF neurons stream “extramurally,” that is on the surface of the hindbrain parenchyma, and CF neurons stream “intramurally,” within the hindbrain parenchyma. Both migrations, whether extra- or intramural, involve extensive circumnavigation of the developing hindbrain from dorsal to ventral as well as distributing anteriorly and posteriorly before assembling into discrete nuclei. Here we summarize the current view of the precerebellar primordium from the molecular genetic perspective of the last decade. This story, however, has its beginnings over one hundred years ago, with the insightful observations, hypotheses, and terminology of neuroanatomists Wilhelm His [1] and Charles Essick [2].

The rautenlippe of the dorsal hindbrain

Through studies of human embryos performed in the late 1890s and early 1900s, His [1] and then Essick [2], identified a territory of dorsal hindbrain neuroepithelium that, while contiguous, was distinct from the rest of the hindbrain neuroepithelium in its morphology, its sustained mitotic activity well into late-stages of embryogenesis, and its seeming deployment of neurons into superficial streams traversing the hindbrain periphery ventrally. His called this dorsal germinal zone the *rautenlippe* [1], or rhombic lip – aptly named because it appears to unfurl from the rhomboid-shaped opening of the fourth ventricle, much like lips to an open mouth. During hindbrain development, the dorsal alar plates remain unfused along the midline, being bridged together by a thin but extensive epithelial roof plate (also referred to as the medullary velum); the large luminal space lying beneath this tented velum becomes the fourth ventricle, and the neuroepithelium lying immediately adjacent is what His defined as rhombic lip. In mouse embryos, the characteristic shape of the rhombic lip that so intrigued His in human embryos becomes pronounced only at late embryonic stages. At earlier time points it is distinguished largely by proximity to the roof plate and by the emergence of tangentially migrating cells.

His and later Essick describe streams of migratory cells seemingly emanating from the rhombic lip, and proposed contributions to ventrally located pontine and olivary nuclei. This proposal was extraordinarily insightful given the limited methodologies and tissue available at that time. Indirect evidence continued to mount in support of a dorsal origin for precerebellar neurons, as revealed by assays including rhombic lip ablations in chick embryos performed in the 1950s [3], as well as isotopic birthdating studies [4] [5-8] and peroxidase labeling studies [9] in rodents performed during the 1960s-1990s. At this point, there seemed little doubt that precerebellar neurons had a dorsal origin; however, the first actual direct evidence was obtained through the use of chick-quail chimeras in the 1990s. Grafting dorsal portions of quail hindbrain neuroepithelium into chick host embryos *in ovo* and tracking the quail-derived daughter cells revealed ventral migrations and the population of ventral nuclei in the pons and medulla [10,11]. This anatomical delineation of the rhombic lip has now been shaped by contemporary genetics, bringing convergence to anatomical and molecular definitions of the precerebellar primordium.

A ‘marriage’ between classical anatomy, contempory molecular genetics, and cell fate results in a new unified view of the precerebellar rhombic lip

Great interest surrounded the quail-chick chimera fate maps [10,11] because they provided the first direct evidence for what was so insightfully hypothesized by His one hundred years earlier [1] – that the dorsal hindbrain area defined as the rhombic lip gives rise to ventrally migrating precerebellar neurons. Moreover, avian grafting experiments began to take on a new level of resolution. They began to be guided by gene expression domains in addition to anatomical landmarks. Avian embryos processed for *in situ* gene expression served as “training” tissue for microdissections and transplantations using live tissue *in ovo* for fate mapping. What emerged from this advance were the beginnings of a molecular definition of rhombic lip territory [10].

While enormously powerful, these avian approaches could only suggest but not prove relationships between molecularly defined progenitor cells and progeny neuron identity. Even the best of hands cannot dissect with the precision of gene expression, especially when expressing and non-expressing cells intermingle and domain boundaries blur, as is common. This obstacle was overcome, at least in part, with the advent of genetic fate mapping [12,13]. For the first time, progenitor cells delineated genetically could be linked directly to later neuronal identity. In this approach, Cre or Flp recombinase expression is targeted to a molecularly defined cell population in the mouse embryo using specific enhancers and transgenesis. The expressed recombinase then modifies a “silenced” reporter transgene, activating reporter expression to mark the targeted progenitor cells. The “activated” reporter transgene is passed on to all progeny cells, marking them as well. Unrelated (recombinase-negative) cells are not marked because the reporter transgene remains in the “silent” configuration. One of the first territories to be examined using this type of genetic approach to fate mapping was the hindbrain rhombic lip (hRL) [14].

In these initial genetic fate mapping studies, regulatory elements from the *Wnt1* gene were used to drive recombinase expression in the dorsal-most zone of neuroepithelium, in territory similar to that defined anatomically as hRL [14] (Figure 1 B). In addition to its spatial restriction, *Wnt1* also offered the benefit of being expressed in a graded fashion within the hRL territory - highest dorsally, lowest ventrally. This feature, when exploited along with two Flp variants of differing recombination efficiency, allowed for the beginnings of partitioning progenitor differences along the DV continuum of the hRL. Two kinds of recombinase transgenic mice were independently employed: one in which *Wnt1* regulatory elements directed expression of the low activity FlpL variant, capable of inducing recombination and thus reporter transgene activation only in progenitor cells expressing high levels of *Wnt1* mRNA [14,15]; a second in which the same *Wnt1* driver sequences were used to express the high (enhanced)-activity variant Flpe, capable of achieving recombination even in low expressers, and thus marking the entire *Wnt1* gradient [15]. The *Wnt1::Flpe*-generated fate map included MF and CF neuron subtypes [15], while CF neuron subtypes were excluded from the *Wnt1::FlpL* fate map [14,15]. These findings were important for at least three reasons: First, they showed directly for the first time in a mammalian model organism that MF and CF precerebellar neurons indeed arise from dorsal neuroepithelium, further validating the hypothesis of His. Second, they pointed to separate progenitor pools for MF versus CF neurons, with MF neuron subtypes arising from *Wnt1* mRNA^{high} cells (dorsal hRL cells), and CF neuron subtypes, from *Wnt1* mRNA^{low} cells (ventral hRL cells), thus arguing against a previously proposed model [3] of a common progenitor cell for MF and CF neurons. Third, they provided evidence that molecular differences among progenitor cells of the hRL correlate strongly with future cell identity, suggesting that aspects of fate specification may be determined within hRL progenitor cells as opposed to solely later time points at or along the route to their final destinations.

This inferred DV partitioning of MF from CF progenitor cells was borne out upon fate mapping a series of smaller domains within the *Wnt1* territory, utilizing subdomains distinguished by expression of specific basic helix-loop-helix (bHLH) transcription factors. (Figure 1). The dorsal-most tip of the hRL, characterized by *Lmx1a*-expressing progenitor cells, was found to give rise to the epithelia of the hindbrain choroid plexus [15-17]. The more ventrally-located territory delineated by *Math1*(mAtoh1)-expressing progenitors was found to produce precerebellar mossy fiber neurons [18]. Progressing further ventral within the hRL are domains demarcated by expression of *Ngn1*, followed by *Mash1*, and then *Ptf1a* (Figure 1 B). The *Ptf1a*-pool produces CF neurons [19]. In particular, it is the portion of the *Ptf1a* progenitor pool that overlaps with *Wnt1* expression and the expression of another bHLH transcription factor, *Olig3* [20][21], as demonstrated by the *Wnt1::Flpe* fate map [15] and, more recently, the *Olig3::creERT2* fate map [20], which account for nearly all ION CF neurons. While it appears that most CF neurons indeed arise from cells with a history of *Ptf1a*, *Olig3*, and *Wnt1* expression [18,20,22][15,19], a caudal subset of CF neurons in the ION subnuclei called the posteroventral and posterodorsal olive may receive contributions from *Ngn1*- and/or *Mash1*-pools – this is because they are fate mapped by a *Wnt1*-*cre* transgenic [23] that appears to reach ventrally through the *Ngn1* domain into *Mash1* but not *Ptf1a* territory [Landsberg and Dymecki, unpublished data] (Figure 1 B). This possibility is supported further by the correlation between expansion of caudal ION populations upon expansion of the *Ngn1*-marked progenitor pool [15]. Thus, the precerebellar primordium appears divided along the dorsoventral axis into discrete molecularly defined pools of progenitor cells (*Lmx1a*, *Math1*, *Ngn1*, *Mash1*, and *Ptf1a* pools), and is fully delimited dorsoventrally by expression of the genes *Wnt1* and *Olig3* (Figure 1 B).

In these examples, the various employed *Wnt1* transgenics generated by pronuclear injection (as opposed to transgene knock-in) – e.g. *Wnt1::Flpe* [15], *Wnt1*-*cre* [23], *Wnt1::FlpL* [14], and *Wnt1::lacZ* [24] (Figure 1) – illustrate an important technical point worth emphasizing. It is helpful to refer to a particular fate map by the recombinase driver line employed (e.g. *Wnt1*-*cre* fate map or *Wnt1::Flpe* fate map, as examples) as opposed to the endogenous locus (*Wnt1* fate map). This is because the transgenics may not always be mapping the same extent of the endogenous domain, especially when gene expression is graded. The ability to capture and map different extents of a molecularly-defined progenitor pool presumably reflects expression level differences stemming from transgene copy-number and chromosomal integration site. By contrast to *Wnt1*, *Olig3* appears more uniform in its expression dorsoventrally within hRL territory [20][21], providing a more straightforward delineation of the DV extent of the precerebellar primordium.

Having mapped DV molecular coordinates for the origin of MF and CF precerebellar neurons, the next challenge was to resolve the anteroposterior (AP) coordinates. Because the expression profiles of *Wnt1*, *Olig3*, *Math1*, *Mash1* and *Ptf1a* span the AP extent of the hindbrain, they, in and of themselves, provide little AP resolution. Thus, an intersectional genetic fate mapping approach [25] was employed that enabled selective mapping of just those *Wnt1::Flpe*-expressing progenitors that reside within individual rhombomeres (r), in other words, reside at different AP levels within the hRL [26]. Intersectional fate mapping within r2, r3 or r5 [26], as well as conceptually from r1 [22] and Farago and Dymecki, unpublished data], showed no marking of the major precerebellar nuclei (PGN, RTN, LRN, ECN, ION), suggesting by subtraction that they originate from the *Wnt1* domain situated in more caudal rhombomeres (r6-r8). Because r6-, r7- or r8-*cre* lines are lacking for intersectional mapping experiments, in utero electroporation of GFP-encoding plasmid DNA to the caudal-most portions of the hRL (caudal to the otic vesicle which resides roughly at the level of r5) was employed as a proxy. Results showed GFP-marked cells populating all the major precerebellar nuclei, as well as the routes traveled [27,28], confirming a caudal (and dorsal) origin for at least most precerebellar neurons. More recently, an r4-*cre* driver

has been developed, and the published mapping data, while not yet exhaustive nonetheless appears negative with respect to precerebellar lineages [29,30]. Thus, this collective set of mapping data places the precerebellum primordium at r6-r8 AP coordinates. Contributions from territory defined as pseudorhombomeres 9-11 is also possible [31,32]; for simplicity here we include that possibility within the general designation of r6-r8.

Genetic fate mapping has thus rendered a new view of the mouse hRL in general, and the precerebellar primordium in particular. That portion of the rhombic lip productive of precerebellar neurons –referred to as the “precerebellar lip” – resides at the AP:DV coordinates *r6-r8:Olig3*, with further refined DV coordinates based on expression of *Math1*, *Ngn1*, *Mash1*, or *Ptf1a*.

By adding gene mutations to the “relationship,” mechanisms for regulating hRL progenitor cell identity and allocation are revealed

Not only does *Math1* expression in the hRL identify MF progenitor cells [18][15], but its activity is necessary for their development – MF precerebellar nuclei are absent in *Math1*-deficient mice [18,33,34]. Interestingly, elimination of *Math1* also results in diminution of normal olivocerebellar tracts, the latter indicative of defective or diminished CF neurons and/or their projections from the ION to the cerebellum [34]. Without the data from genetic fate maps, it was unclear whether or not these CF defects reflected a cell autonomous requirement for *Math1* in CF neurons in addition to MFs, for example for CF axon extension and/or maintenance. Genetic fate mapping determines this not to be the case given that the CF lineage showed no history of *Math1* expression, therefore, CF defects in *Math1* null mice must be secondary. Thus genetic fate maps and phenotypic analyses following targeted gene loss inform and advance each other in important and powerful ways.

Indeed, through a similar series of studies, *Ptf1a* has been shown to be required cell-autonomously for the development of most, if not all, CF neurons [19]. More specifically, in the absence of *Ptf1a* protein, newly postmitotic CF precursors appear to accumulate laterally, lose expression of CF markers (e.g. the transcription factors *Brn3a* and *Brn3b*) and undergo apoptosis [19]. *Ptf1a* nulls also show abnormal population of some MF nuclei with *Ptf1a*-descendant neurons. This is striking because *Ptf1a*-expressing progenitors do not normally give rise to MF neurons – that is of course a task typically reserved for *Math1*-descendants. Thus *Ptf1a* appears required to suppress hindbrain MF fate specification programs. It will be interesting to assess whether *Math1* expression is upregulated in aspects of the hindbrain *Ptf1a*-progenitor pool in the absence of functional *Ptf1a* protein, offering a possible explanation for the MF finding. Indeed, there is precedent for such a fate switch in the cerebellum, where *Ptf1a*-deficiency results in loss of *Ptf1a*-descendant Purkinje cells [19,35] and gain of *Math1*-descendant granule cells [35]. Further extending this parallel, Purkinje cells and granule cells are target populations for CF and MF neurons respectively.

There are other transcription factors expressed in the hRL which serve patterning rather than strict fate specification roles, one example being the homeodomain-containing protein *Pax6*. Dorsal *Pax6* expression is unique to the hindbrain as compared to the spinal cord [15]; in the latter, *Pax6* expression is limited to the basal plate only. This wave of dorsal *Pax6* expression in the hindbrain coincides temporally and spatially with precerebellar neurogenesis [15] and functionally appears to influence the relative extent of hRL DV subdomains, expanding certain progenitor pools at the expense of others, and ultimately skewing the relative proportion of neuron types in the mature brainstem [15]. More specifically, in *Pax6* deficient embryos, the *Math1* progenitor pool in the precerebellar lip appears reduced while the *Ngn1* pool and possibly others appear expanded. Commensurate with these changes and the fate map results, these mice show reduction of MF nuclei and

enlargement of the caudal portions of the ION CF nucleus [15]. Pax6 deficient embryos also show reduction of *Msx* gene expression in the precerebellar lip. This collection of findings suggests a possible mechanism. Pax6 may pattern the precerebellar lip by potentiating the effects of secreted bone morphogenetic proteins (BMPs). In the absence of this potentiation, the dorsal-to-ventral BMP signaling gradient within the hRL may be lessened, resulting in the diminished induction of *Math1* expression (which is thought to require high levels of BMP signaling [36]) yet expanded induction of *Ngn1* expression (which is thought to require low BMP signaling). Further solidifying the Math1/Ngn1 border would be their repression of each other. *Msx* gene expression would be reduced because they are direct transcriptional targets of BMP signaling.

Olig3 is another critical hRL factor, involved in patterning and specification events. Loss of Olig3 results in reduction of the Math1 and Ngn1 domains and expansion dorsally of the Ptf1a territory [20,21]. As expected given the Math1 reduction, MF nuclei were reduced. Interestingly, most CF neurons are also lost in the absence of Olig3, despite the dorsally expanded Ptf1a pool and that CF neurons arise from Ptf1a-expressing progenitors. The expanded Ptf1a pool instead generates Lbx1-positive postmitotic neuronal precursors similar to those generated from the more ventrally located Olig3-negative portions of the Ptf1a territory [20,21]. Thus, a CF fate appears to require both Olig3 and Ptf1a. Consistent with this model, it is only upon electroporation of Ptf1a and Olig3 expression vectors, but not either alone, that Foxd3-positive CF precursors are generated [20].

The Nuclear Factor I (Nfi) – type transcription factor, Nfib, acts in yet a different way within the precerebellar lip. Nfib appears to regulate either the rate of neurogenesis or the rate of migration from the lip [37] while leaving patterning intact. As a consequence, formation of MF nuclei is delayed by a few days.

Generating diversity within a precerebellar system

An important next challenge lies in delineating how specific subtypes within the mossy or climbing fiber systems are determined. One mechanism recently uncovered appears to involve heterodimers between Math1 and the E-protein, Tcf4 [38]. Mice deficient in Tcf4, and thus lacking in Math1/Tcf4 heterodimers, show a selective deficit in pontine but not medullary mossy fiber nuclei; CF neurons also are unaffected. Interestingly Tcf4, like Math1, appears to be expressed in all MF progenitors in the precerebellar lip, yet is required for proper development of only the pontine MF subclass. Thus there appear to be two classes of MF progenitors one dependent upon Tcf4 and the other not. Dependence may involve other transcriptional cofactors that require Tcf4 for dimer interaction and ultimately for driving specific differentiation programs.

The bHLH transcription factors NSCL-1 and NSCL-2 are expressed in newly born (postmitotic) MF neurons as they migrate away from the hRL to ventral territories [39]. Like the case for Tcf4, mice deficient for NSCL-1/2 show defective migration of just the pontine MF neuron subclass. Thus Math1, Tcf4, and NSCL-1/2 may be part of a larger regulatory cascade distinguishing and determining pontine MF fate.

Recent work on characterizing the Linear nucleus (Li) has demonstrated how the convergence of anatomical and genetic fate mapping studies leads to a clearer picture of hindbrain organization. The Linear nucleus was shown anatomically to extend from the LRN, to project to the cerebellum, express known precerebellar markers, and descend from both Math1 and Wnt1 expression domains, firmly placing it in the precerebellar system [40].

Conclusions

Emerging from this collective body of work is a new definition of the precerebellar primordium and the hindbrain rhombic lip – notably, one that furnishes convergence of anatomical and molecular parameters. In this definition, *Olig3* expression delimits and defines what is called the hindbrain rhombic lip. The *Olig3* territory, by contrast to that of *Math1* [15,18,20,22] for example, maintains consistency with the classical neuroanatomical definition of the rhombic lip as giving rise to both major precerebellar systems, mossy and climbing. Furthermore, *Olig3* is not only expressed in both mossy and climbing fiber progenitors but it is required for the proper formation of both systems. Also defined by the summarized studies is that the precerebellar section of the hRL restricts to that portion of the *Olig3* territory that resides within r6-r8, giving the “precerebellar lip” the AP:DV coordinates *r6-r8:Olig3*. DV position within the *Olig3* domain refines precerebellar progenitor pools further based on expression of the bHLH-encoding genes *Math1*, *Ngn1*, *Mash1*, and *Ptf1*. These proneural genes, especially *Math1* and *Ptf1a*, not only mark distinct MF and CF progenitor pools respectively, but also are required for their proper specification. By contrast, *Pax6* functions are modulatory influencing patterning of the lip and the proper allocation of neuroepithelial cells amongst these molecularly distinct progenitor pools. Finally, layered upon these patterning and specification programs are additional bHLH factors, like *Tcf4* and *NSCL-1/2*, which enable the development of diversity within a given system. His and Essick, it seems, would be astounded by the elegant molecular dances enacted within the rautenlippe.

Reference Annotation: Of outstanding Interest

- [1] A foundational neuroanatomical work describing the human rhombic lip and presumptive precerebellar migrations. This work remains relevant as the neurodevelopmental research community attempts to build a consensus between the anatomical and molecular definitions of the rhombic lip.
- [15] This study further molecularly defines and subdivides the dorsal – ventral subdomains of the precerebellar lip. Though these domains were based on the graded expression of a transgene, the subdomains were later correlated with the expression of genes such as *Atoh1*, *Ptf1a* and *Olig3* which are all required for the proper development of the precerebellar nuclei
- [18] *mAtoh1* (*Math1*) descendants are mapped by the per-durance of LacZ, showing critical contributions several precerebellar nuclei and delineating their migratory pathways. Further loss of function for *mAtoh1* showed a requirement for *mAtoh1* in the development of portions of the precerebellar system.
- [26] Rhombomeric specific cre drivers are combined with a *Wnt1::Flpe* driver to intersectionally mark the descendants of rhombic lip r2, r3 and r5. This work combined DV fate mapping [15][18,22] and electroporation studies [27][28] to localize the precerebellar rhombic lip progenitor domain to the upper quarter of rhombomeres r6-r8 along the A-P axis.
- [20] *Olig3* endogenous expression, fate mapping and loss of function phenotype suggest it is currently the most definitive molecular marker for the rhombic lip. *Olig3* expression and fate mapping covers domains that give rise to precerebellar neurons and loss of *Olig3* disrupts progenitor specification and ultimately shows later defects in precerebellar nuclei.

[21] This loss of function and expression analysis study for *Olig3* demonstrates a clear requirement for the development of the precerebellar system and its expression in precerebellar progenitor domain.

[19] In addition to describing the requirement of *Ptf1a* for the development of the ION, it clarified cell autonomous vs cell non-autonomous requirements for proper ION and mossy fiber nuclei development in the cerebellar mutant through the use of fate mapping [42].

[40] This work shows the importance of both anatomical and molecular studies in characterizing hindbrain development. Precise anatomical, retrograde tracing and genetic fate mapping studies clearly define the Linear nucleus as part of the precerebellar system.

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Fig. 1.

A) A schematized mouse hindbrain concurrently representing multiple time points in development. The rhombic lip in purple is shown to frame the opening of the 4th ventricle. Within the presumptive rhombic lip, the anterior - posterior domain of the precerebellar neuron progenitor zone was uncovered by fate mapping of multiple rhombomeres (r) and electroporation experiments that localized the precerebellar domain to r6 - r8. Based on further fate mapping studies, we can now place the precerebellar germinative neuroepithelium in the dorsal half of the alar plate of r6 - r8. The precerebellar progenitor domain then gives rise to three migratory streams. Two are mossy fiber streams (yellow), the anterior extramural stream (AES), which gives rise to the Pontine Nucleus (PN) and Reticular Tegmental Nucleus (RTN); and the posterior extramural stream (PES) which gives rise to the External Cuneate (ECN) and Lateral Reticulate Nucleus (LRN). The third stream (green), the intramural migratory stream (IMS), gives rise to the Inferior Olive Nuclei (ION). Initial ipsilateral and contralateral projections of all precerebellar nuclei are shown in red as they begin migrations toward the cerebellum.

B) A schematized cross section through mouse rhombomere 7 at embryonic day 11.5 (E 11.5). The precerebellar neuron progenitor domain was first molecularly defined by various *Wnt1::recombinase* transgenes. The variability in ventral expression of different *Wnt1* transgenes delineated a separation in the mossy fiber (MF, yellow) and climbing fiber progenitor domains (CF, green). These dorsal - ventral progenitor domains (dark purple, da1 - da4) [41] were further defined along the DV axis by genetic fate mapping with other markers including *mAtoh1*, *Mash1*, *Ptf1a*, and *Olig3*. *Olig3* endogenous expression, fate mapping and loss of function phenotype suggest that *Olig3* is currently the most definitive molecular marker for the hindbrain rhombic lip. Other abbreviations, VZ - Ventricular Zone, MZ - Mantle Zone, CPe - Choroid Plexus epithelium,