

Math-Map(ic)s

In this issue of *Neuron*, Wang et al. and Machold and Fishell present contrasting molecular fate maps of *Math1*, which redefine the derivatives of the embryonic rhombic lip and offer a conceptual overhaul of cerebellar and precerebellar development. These fate maps identify a common developmental thread linking diverse, functionally associated neurons and reveal an exquisite temporal organization in cell production within a precise spatially defined region of neuroepithelium.

Fate maps remain one of the most important tools in the arsenal of experimental techniques available to the developmental biologist. The most prized outcome of a fate map is a simplified relationship between embryonic organization and adult function. Recently, transgenic approaches in mouse have reinvigorated this approach by allowing fate maps to be generated by molecular expression. These can reveal unexpected developmental homologies between disparate structures and illuminate the role of genes in development. These aspects of the molecular fate map are exemplified by two papers in this issue of *Neuron*, which use different strategies to generate maps of derivatives of cells that express *Math1* at the embryonic rhombic lip (Machold and Fishell, 2005; Wang et al., 2005). Viewed alongside recently published work in this journal (Hoshino et al., 2005), they substantially simplify our understanding of the patterning processes underlying cerebellar and precerebellar development and illustrate the mechanisms that generate neural diversity in the early neural tube.

The rhombic lip comprises the interface between the nonneuronal roof plate of the fourth ventricle and the neural tube (Figure 1A). For over a hundred years, it has been recognized as the likely source of migratory neurons that stream away from the roof plate to form a range of nuclei in the adjacent cerebellum and hindbrain. The most prominent of these is the external granule-cell layer (EGL), a unique secondary germinal epithelium, which accumulates over the developing cerebellum and which gives rise to cerebellar granule cells. A consensus view emerged that granule-cell precursors alone were generated from the cerebellar (upper or rostral) rhombic lip. By contrast, the hindbrain (lower or caudal) rhombic lip gave rise to precerebellar nuclei, which project mossy fibers to granule cells, and the inferior olive, which projects climbing fibers to cerebellar Purkinje cells (Harkmark, 1954). Even these relatively rudimentary observations presented the intriguing possibility that a functionally connected system developed according to common rules from a single epithelium. Ironically, the advent of more sophisticated quail-chick microsurgical fate mapping and retroviral techniques presented somewhat contradictory results that muddled these earlier interpretations. These were partially resolved by the advent of molecular approaches. Gene-

expression patterns facilitated revised fate maps, and the analysis of mouse mutants reasserted that the rhombic lip comprises a defined epithelium organized into different pools along the rostrocaudal axis (Wingate, 2001). Most notably, the shortcomings of conventional lineage and grafting approaches were comprehensively circumvented by an innovative molecular fate-mapping approach, which demonstrated that hindbrain rhombic-lip derivatives expressing *Wnt1* give rise to mossy-fiber projecting neurons (Rodríguez and Dymecki, 2000). Of all the candidate patterning genes expressed at the rhombic lip, the bHLH transcription factor *Math1* (*Atoh1*) appears to lie closest to the top of the hierarchy of rhombic-lip specification (Figure 1A). *Math1* is the vertebrate ortholog of the proneural *Drosophila* gene *atonal*, which specifies proprioceptive chordotonal organs (Bertrand et al., 2002). In the rhombic lip, a null mutation in *Math1* stalls both precursor differentiation and migration, leading to a complete loss of the cerebellar granule-cell layer and hindbrain mossy-fiber afferent nuclei.

The two papers published in this issue of *Neuron* have used different approaches to generate a molecular fate map of the derivatives of *Math1*-positive precursors at the rhombic lip. Machold and Fishell have generated a line of transgenic mice where *Math1*-positive precursors and their derivatives are immortally labeled on transient exposure to tamoxifen. This enables discrete temporal cohorts to be identified at a number of successive time points (Figure 1B). Further, they use a transgenic *Engrailed-1* mouse line (*En1-Cre*) to determine which derivatives are exclusive to rhombomere 1 (*En1*-positive, *Math1*-positive precursors) and thus derivatives of the cerebellar rhombic lip, borrowing conceptually from the powerful new technique of transsectional genetics (Awatramani et al., 2003). Wang et al. perform a detailed comparison of β -galactosidase expression driven by *Math1* in normal and null-mutant mice, relying on the perdurance of protein in rhombic-lip derivatives to produce a comprehensive, cumulative map of *Math1*-dependent nuclei in both the cerebellum and hindbrain (Figure 1B). Both papers concur on the identity and birthdates of *Math1* derivatives where their results overlap. Together, they offer a radical revision of the role of the rhombic lip in cerebellar and precerebellar development and reveal a detailed temporal organization in cell production from a spatially defined neural epithelium.

Reevaluating the Output of Rhombic Lip

The core circuit of the cerebellum is very simple: excitatory inputs to Purkinje cells are either direct (climbing fibers) or mediated by granule cells (from mossy-fiber input). Similarly, inhibitory Purkinje-cell output is either direct (to the vestibular nuclei) or, for the most part, indirect to the thalamus, red nucleus, and vestibular and reticular nuclei via the deep cerebellar nuclei. An unanticipated finding of both *Math1* studies is that these deep cerebellar relay neurons are, like granule cells and mossy-fiber nuclei, rhombic-lip derivatives (Figure 1C). From their basal position and nuclear orga-

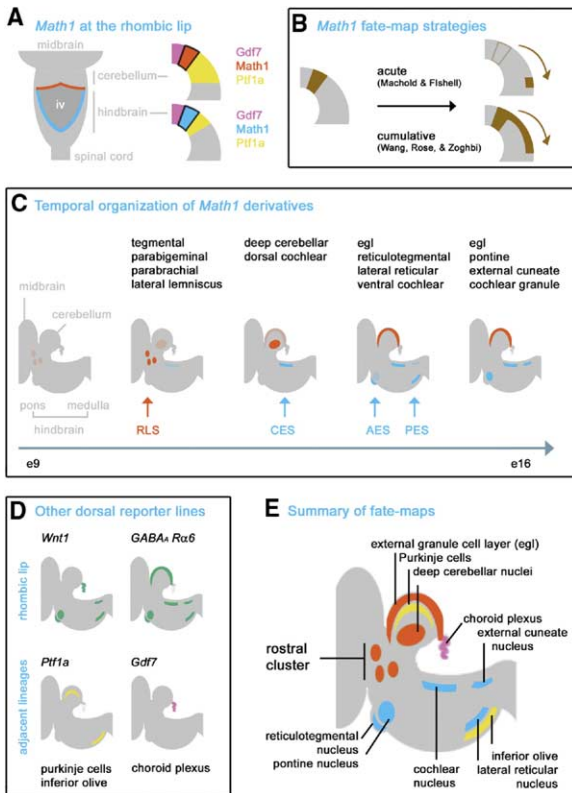


Figure 1. Dorsal Lineages Contributing to the Cerebellar and Pre-cerebellar Systems

(A) In a schematic dorsal view of an early (E9.5) embryo, *Math1* expression characterizes the full extent of the cerebellar (red) and hindbrain (blue) rhombic lip and defines the edge of the roof plate of the fourth ventricle (iv). (B) Two different approaches to molecular labeling generate either an acute (Machold and Fishell, 2005) or cumulative (Wang et al., 2005) fate map of rhombic-lip derivatives. While both maps concur on the identity and timing of neuronal production, an acute label demonstrates that *Math1* expression is transient and also characterizes cells that transit out of the rhombic lip. (C) Derivatives of each of the defined migratory streams identified from the rhombic lip by Wang et al. (RLS, CES, AES, and PES) are chronotopically distributed. In longer migration paths, the furthest (most distal) derivatives from the rhombic lip are generated first. Details of the development (E11 onward) of the cochlear-nucleus development (CES) are not shown. (D) Other molecular fate maps have identified subsets of the *Math1* rhombic-lip lineages. Cerebellar and cochlear derivatives are not a product of the *Wnt1*-positive rhombic-lip precursor pool (Awatramani et al., 2003; Rodriguez and Dymecki, 2000). Adjacent neuroepithelia give rise to closely related structures. *Ptf1a* identifies the precursors of Purkinje cells and the inferior olive (Hoshino et al., 2005). *Gdf7* expression defines precursors of the choroid plexus (Curlle et al., 2005). (E) A summary composite of fate maps reveals how a diversity of functionally related structures is generated by spatiotemporal patterning of the dorsal neural tube. RLS, rostral rhombic lip; CES, caudal rhombic lip; AES, anterior extramural stream; PES, posterior extramural stream. This figure was prepared with a colorblind barrier-free color pallet (<http://jfly.iam.u-tokyo.ac.jp/color/>).

nization, it seemed “beyond doubt that the [deep] cerebellar nuclei must be derived from the ventricular matrix” (Harkmark, 1954), and all studies to this point have been interpreted in this light. One such recent paper (Hoshino et al., 2005) describes the lineage of precu-

sors expressing the *cerebellless* gene, which encodes the bHLH protein *Ptf1a* (Figure 1D). When this fate map is viewed alongside the *Math1* fate maps, the lineage of cerebellar neurons becomes radically simplified. Inhibitory neurons (Purkinje, basket, and Golgi cells and small deep cerebellar neurons) and excitatory neurons (granule cells and large deep cerebellar nuclei) are born in mutually exclusive dorsal domains specified by their expression of *Ptf1a* and *Math1*, respectively (Figure 1E). The relatively simple cellular structure of the cerebellum reflects an earlier simple dorsoventral code (Figure 1A), which becomes obscured by the subsequent migration of rhombic-lip derivatives.

The two *Math1* fate maps offer further important revisions to our view of rhombic-lip derivatives. Various rostral hindbrain nuclei, including a population of cholinergic neurons (Machold and Fishell, 2005) are also derived from cerebellar rhombic lip. All these rostral nuclei are explicitly linked to a vestibular/auditory/cerebellar system. In the hindbrain, the ventral cochlear nucleus and granule cells of the dorsal cochlear nucleus are identified as *Math1* derivatives (Wang et al., 2005), while the absence of label in the inferior olive corresponds with its identification as a derivative of the *Ptf1a* precursor pool (Figure 1D). Overall, the list of *Math1*/rhombic-lip derivatives now includes not only cerebellar granule cells and their precerebellar (mossy-fiber) inputs, but also deep cerebellar nuclei and various elements of a broader proprioceptive pathway. By comparison, the direct climbing-fiber circuit of the inferior olive and its target cerebellar Purkinje cells appears to be derived from a parallel pool of *Ptf1a*-positive neurons lying close to the rhombic lip (Figure 1E).

Introducing a New Order

Generating diversity by the spatial parcellation of the neural tube into increasingly smaller uniquely coded molecular domains is a theme that has been widely explored over the last 10 years. A single parcel of neuroepithelium can itself generate a diversity of derivatives through temporal patterning. The *Math1* fate maps demonstrate a precise temporal structure to the production of rhombic-lip derivatives and for the first time establish the identity of extracerebellar derivatives of the cerebellar rhombic lip reported in other species. As in the avian and zebrafish embryo, nuclei distal to the rhombic lip appear to be born first (Figure 1C), suggesting that derivatives migrate progressively shorter distances with time—a phenomenon that has been observed at a much finer scale within the EGL and that is confirmed here (Machold and Fishell, 2005). This progressive change in migration capacity produces a chronotopic arrangement of neurons correlating with cell fate: cholinergic cerebellar rhombic-lip derivatives are among the first born, while later cohorts both are glutaminergic and, by comparison with another reporter line (Fünfschilling and Reichardt, 2002), express the $\alpha 6$ subunit of the GABA $_A$ receptor (Figure 1D).

A question for future studies will be how this temporal organization is regulated. One factor is the changing competence of a precursor pool to respond to inductive signals from the roof plate that specify *Math1* expression. A further possibility is a change in inductive cues, which are generated from the rhombic lip itself. The origin of both rhombic-lip and roof-plate lineages

lies within the *Wnt1*-positive precursor pool (Awatramani et al., 2003; Rodriguez and Dymecki, 2000). These can, however, be segregated on the basis of *Math1* and *Gdf7* expression, respectively (Figure 1D). While *Math1* derivatives are neuronal, the *Gdf7* lineage gives rise to nonneuronal roof plate, which in turn generates the choroid plexus (Curre et al., 2005), an important late source of retinoic acid that matures in parallel with the rhombic lip. The proximity of the two precursor pools is illustrated by the fine balance between rhombic-lip and roof-plate fate. In the *Math1* null mutant, significantly more *Math1*^{-/-} precursor cells enter the spinal cord roof plate (Bermingham et al., 2001).

The Function of *Math1*

These studies demonstrate the power of molecular techniques to map precursor fate but also shed light on the role of *Math1* both at the rhombic lip and more widely within the developing embryo. The technically innovative approach of Machold and Fishell presents an intriguing observation: *Math1* expression is transient within rhombic-lip precursors, and cells that express *Math1* rapidly transit out of the rhombic lip, leaving behind no residual precursor population. Only granule-cell precursors of rhombomere 1 retain the capacity for cell division. This confirms a growing body of evidence that *Math1* initiates cells into a program of differentiation but has a much reduced proneural function within the CNS (Bertrand et al., 2002). Machold and Fishell highlight the important question of how the *Math1*-positive rhombic-lip precursor pool is replenished. Two possibilities present themselves: either there is a resident, self-renewing *Math1*-negative precursor pool at the rhombic lip, or precursors are derived from surrounding epithelia. The former implies an as yet undiscovered rhombic-lip stem-cell lineage feeding into the *Math1* precursor pool. The latter explanation would mean that the rhombic lip is a dynamic zone of *Math1* induction, drawing in precursors from a spatially adjacent epithelium, with the onset of expression concomitant with both cell specification and the initiation of a program of migration. Whichever the explanation, this conundrum begs a closer investigation of microanatomical molecular organization and dynamics of cell division at the rhombic lip.

From their comprehensive analysis, Wang et al. propose that *Math1* unites a range of disparate cell groups into a common functional pathway—proprioception. In its strictest definition, this can be seen as the set of sensations pertaining solely to the musculoskeletal system as transmitted by a range of deep receptors and spinal interneurons, whose differentiation has been shown to require *Math1* (Bermingham et al., 2001). However, in Charles Sherrington's original formulation of proprioception, the vestibulocochlear reflexes that also regulate balance and posture were unambiguously included with spinal proprioceptors, since they “appear to cooperate together and form functionally one receptive system” (Sherrington, 1906). While only certain elements of classical vestibular nuclei are labeled in *Math1* fate maps, Wang et al. state a convincing case that spinal proprioception and the vestibular/auditory/cerebellar system are united by a common developmental

theme. Somewhat unexpectedly, this goes some way to reviving Sherrington's definition of the cerebellum as the coordinating center, or head ganglion, of the proprioceptive system. It also points to an evolutionary conservation of atonal function in vertebrate and flies as the gene for proprioception.

Do these molecular approaches to lineage tracing sound the death knell of conventional fate mapping? The resolution of expression maps and their evident power to reveal meaningful genetic homologies between disparate structures would seem to argue so. However, the data obtained by genetic approaches are fundamentally different from fate mapping based on dye injection or chimeric approaches. What is clear from a comparison of the two studies in this issue is that *Math1* expression does not identify a spatially defined precursor pool as such, but rather a specific time point along the path of determination within these precursors. Thus, we now have a complete description of the cellular output of this *Math1* pathway; however, the nature of the ultimate progenitors of the rhombic lip, which continually replenish the supply of *Math1* cells, remains elusive for the time being. While fate mapping genetic lineages might not equate precisely to fate mapping embryonic structures, Machold and Fishell show how a conditional approach can address, in a novel way, the temporal coding within a defined precursor pool. It is reasonable to conceive of time as representing the third axis in a Cartesian model of neural patterning where anteroposterior and dorsoventral positional values provide a framework of spatial coordinates. The ability to explore this extra dimension raises the intriguing possibility of discovering whether a unique spatiotemporal address can be assigned to the origin of each of the classes of neurons in the central nervous system.

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