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Specification and Differentiation of Cerebellar GABAergic Neurons

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Abstract Cerebellar GABAergic projection neurons and interneurons originate from the ventricular neuroepithelium of the cerebellar primordium. However, while projection neurons are born within this germinal layer, interneurons derive from progenitors that delaminate into the prospective white matter. In spite of this common origin, the two main classes of GABAergic neurons are generated according to distinct strategies. Projection neurons are committed to their fate at early ontogenetic stages and acquire their mature phenotypes through cell-autonomous mechanisms. On the contrary, the different categories of cerebellar interneurons derive from a single pool of multipotent progenitors, whose fate choices, production rates and differentiation schedules are strongly influenced by environmental cues.

Keywords Neurogenesis · Neuronal differentiation · Ptf1a · Pax-2 · GABAergic interneuron · Projection neuron

All cerebellar GABAergic neurons originate from progenitor cells that proliferate in the ventricular neuroepithelium (VN) of the cerebellar anlage and express the transcription factor Ptf1a, which is required to specify the GABAergic identities vs the glutamatergic fate [1]. Among the VN-derived types, projection neurons (Purkinje cells and nucleo-olivary neurons) are born at the onset of cerebellar neurogenesis, by progenitors residing within the germinal layer [2]. On the other hand, GABAergic interneurons are produced during late embryonic and postnatal development, following a precise inside-out sequence according to their position in the deep nuclei or laminar placement in the cortex [3, 4]. The earliest-born interneurons (e.g. those of the deep nuclei) also originate from progenitors that reside in the VN. Later on, however, these progenitors delaminate into the prospective white matter (PWM), where they continue to divide and give rise to the different categories of cerebellar inhibitory interneurons.

The mature identities of projection neurons are established in VN cells by the expression of specific combinations of transcription factors. Accordingly, heterochronic/heterotopic transplantation experiments indicate that committed precursors to such phenotypes acquire mature traits in a cell-autonomous manner, regardless of the recipient environment [5]. On the other hand, similar experiments indicate that the different categories of interneurons derive from a common pool of naïve multipotent progenitors, whose fate choices are dictated by environmental cues [3, 4]. As consequence, following transplantation these cells adopt host-specific phenotypes.

GABAergic interneurons are characterised by the transitory expression of Pax-2 [6], which is activated at the time of their last mitosis [4], under the influence of the transcription factor Ascl1 [7]. Before migrating to their final location, the young postmitotic interneurons sojourn for several days in the PWM, where they progress in their maturation and make their final phenotypic choice. Indeed, early postmitotic interneurons transplanted to heterochronic/heterotopic recipients are still able to switch their fate and adopt host-specific identities [4]. Together, these observations indicate that PWM progenitors are committed to the GABAergic inter-

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neuron fate at the time of their last cell division, when Pax-2 is switched on. The young postmitotic cells, however, retain the potential for generating the whole repertoire of cerebellar interneurons and acquire specific mature identities under the influence of instructive cues provided the surrounding microenvironment.

The distinct subpopulations of cerebellar interneurons are considerably different in size. As a consequence, the quantities of different types of interneurons that are generated must be also finely regulated. Such a mechanism should modulate the production rates of interneurons at different ontogenetic stages in order to allow the generation of desired amounts at due times. Hence, analysis of cell proliferation in the PWM reveals a brisk increase in the mitotic rates during the first postnatal week, at the time when molecular layer interneurons, by far the largest interneuron subtype, are generated [8].

The control of interneuron numbers is operated through the concurrent action of cell-autonomous mechanisms and extrinsic factors. On one side, a number of environmental molecules, such for example Sonic Hedgehog [9] or thyroid hormones [10], stimulate the proliferation of interneuron progenitors. On the other, intrinsic regulators of cell cycle dynamics, such as cyclin D2 [8], are required to sustain accelerated mitotic rates. Interestingly, loss of cyclin D2 [8], as well as other cell cycle regulators such as *Myc* genes [11], also delays the maturation of postmitotic interneurons, suggesting that events occurring in dividing progenitors influence the course of subsequent developmental processes.

On the whole, these findings indicate that cerebellar GABAergic projection neurons and interneurons are generated according to distinct strategies. Projection neurons are generated within the VN itself and their developmental schedules are firmly established at the time of their birth. On the contrary, the generation of the variety of interneurons appears to be largely dependent on extrinsic influences, so that their types and numbers can be adjusted in response to specific demands that may arise during the course of cerebellar development. Given the protraction of cerebellar ontogenesis during postnatal life, the latter mechanism governing the genesis of interneurons and the ensuing shaping of local circuitries may result particularly suitable to sustain neural adaption to experience-dependent constraints.

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Conflict of interest We declare here that no conflict of interest of any sort exists for both authors, Ketty Leto and Ferdinando Rossi, of this article.

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