DIVERSIFICATION OF VENTRAL INTERNEURON POPULATIONS IN THE DEVELOPING SPINAL CORD

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Initiation of movements and control of body posture depend on multiple interconnected neuronal networks located in the cortex and in other regions of the encephalon. The activity of these networks eventually converges toward the primary motor cortex wherein primary motor neurons project to multiple nuclei and to the spinal cord and innervate local spinal motor circuitries. Interestingly, whereas cortical networks ensure the initiation of any motor activity, local spinal circuitries autonomously regulate stereotyped aspects of movement including speed, flexor/extensor alternation, rhythmicity and left/right alternation during locomotion.

Spinal motor circuits are composed of motor neurons and of dozens of different premotor interneuron types located in the ventral horn of the spinal cord. Most of these adult premotor interneuron populations arises during development from 4 cardinal classes of embryonic ventral interneuron populations, which progressively diversify into discrete subsets with specific properties and activity. However, the correspondence between these embryonic populations and the dozens of interneuron types described in the adult spinal cord remains elusive.

Therefore, we undertook to characterize novel subsets of ventral interneurons in the embryonic spinal cord. Using an improved labeling technique on whole-mount spinal cords, we demonstrated that these subsets are non-homogeneously distributed along the anteroposterior axis, likely related to their specific contribution to the motor circuitries (Fig. 1). This comprehensive molecular profiling of ventral interneurons provides an important resource for investigating neuronal diversification in the developing spinal cord and for refining our knowledge of the function of each neuronal subset in motor control (2). We also contributed to the discovery of late subsets of V0 and V2 interneurons (5).

![Fig. 1: Anteroposterior distribution of ventral interneuron subpopulations in the developing spinal cord.](image)

Using whole-mount immunofluorescence, we characterized the relative distribution of all the ventral interneuron populations (vIN) and of specific ventral interneuron subsets (here the V0, V1, V2 and V3 populations) in different portions of the embryonic spinal cord (here in the lumbar region). Immunolabeling for Foxp1, which is present in the lateral motor columns, enable to delineate the brachial and lumbar regions.
The transcription factors of the One-cut family, namely HNF-6, OC-2 and OC-3, are transiently expressed in all neurons at the onset of neuronal differentiation. At later stages, they become restricted to multiple well-defined or diffuse neuronal populations dispersed throughout the encephalon and the spinal cord. In the ventral spinal cord, One-cut factors are present in subsets of motor neurons and of each population of ventral interneurons. We recently observed that One-cut proteins are necessary for the diversification of several ventral interneuron populations, as we previously demonstrated for motor neurons (1,8). Indeed, in the absence of One-cut factors, some interneuron subsets are not generated. In addition, we uncovered that these factors are required for proper migration of different interneuron populations (Fig. 2). Current investigations aim at identifying the molecular mechanisms whereby One-cut proteins regulate differentiation and migration in the ventral spinal cord.

Furthermore, we observed that neuronal subsets wherein One-cut factors are not detected in wildtype embryos are altered in One-cut compound mutant embryos. This suggests that these transcription factors control a non-cell autonomous mechanism involved in proper spinal cord development. Conditional inactivations of the One-cut genes are in progress to identify the population(s) that mediate this non-cell autonomous control and to determine the molecular mechanisms implicated in this process.

Fig. 2: One-cut factors control the migration of ventral spinal interneurons.
Top: the location of the V2a interneurons, characterized by the presence of the transcription factor Chx10, is altered in the absence of One-cut factors. Bottom: a Matlab routine has been adapted to analyze the relative position of any neuronal population in the spinal cord from a set of successive sections and to determine whether this distribution is statistically abnormal.
IDENTIFICATION OF A NOVEL POPULATION OF VENTRAL SPINAL INTERNEURONS

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Our expression studies of the Onecut factors in the developing spinal cord unveiled the existence of a yet uncharacterized population of ventral spinal interneurons. These cells start to be generated at very early stages from the p2 progenitor domain. The size of this population is similar to that of the two other major neuronal subsets that arise from this same progenitor domain, namely V2a and V2b. However, they are distinct from these cells in terms of molecular markers, migration pattern and final location. They were temporarily named V2x interneurons. Furthermore, it has been demonstrated that proper generation of V2a and V2b interneurons depends on a binary cell fate decision mediated by the Notch signaling pathway and on different transcription factors including Ascl1 and Foxn4. In contrast, the production of V2x cells is not altered in Ascl1 or Foxn4 mutant embryos nor in embryos deficient in the Notch pathway. Therefore, the developmental determinants of the V2x cells are different from those involved in the generation of other V2 subsets. Hence, we have discovered a yet unknown population of ventral spinal interneurons.

We have identified one specific marker of the V2x interneurons. We are currently generating loss-of-function and gain-of-function models to understand the role of this factor in these cells. In addition, we are using intersectional genetics to specifically target these cells during embryonic development and unravel their progeny, their connectivity, their specific properties and their roles in motor control.

ONECUT FACTORS CONTROL THE DEVELOPMENT OF DORSAL INTERNEURONS

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As observed in the ventral spinal cord, Onecut factors are also present in the developing dorsal interneurons, most of which eventually colonize the dorsal horn of the spinal cord to constitute primary relays for somatosensory information in their transit from the periphery towards the encephalon while others migrate ventrally to eventually integrate into the premotor circuitries. The distribution of Onecut proteins in the dorsal interneuron populations displays a particular spatial and temporal regulation. Indeed, these factors are absent from the dorsalmost populations while their prevalence increases progressively in more ventral cells. In addition, their expression is transient in dorsal populations but progressively more persistent in ventral cells. This suggests that the expression of Onecut factor might be regulated by morphogen gradients or signaling pathways involved in the spatial and temporal patterning of the developing spinal cord (Kabayiza et al., in preparation).

Furthermore, we uncovered that Onecut factors are involved in multiple aspects of the development of dorsal spinal interneurons. In some subsets, Onecut regulates the size of the neuronal population, likely acting in a non-cell autonomous manner on the exit of these cells from the cell cycle. In other populations, Onecut proteins contribute to the diversification of dorsal interneurons by promoting the differentiation into a particular subpopulation at the expense of the others. Finally, Onecut factors control ventral migration of the premotor interneurons that originate from the dorsal spinal cord (Kabayiza et al., in preparation). Experiments are ongoing to identify the molecular mechanisms whereby Onecut exert these different roles in dorsal spinal interneurons.
ONECUT FACTORS REGULATE FINAL REORGANIZATION OF THE PURKINJE CELLS IN POSTNATAL CEREBELLUM DEVELOPMENT

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We previously noted that adult mice single mutant for the Hnf6 gene exhibit locomotion defects characterized by hindlimb muscle weakness, abnormal gait and defective balance and coordination. Indeed, HNF-6 is required in spinal motor neurons for proper formation of the hindlimb neuromuscular junctions, which likely explain muscle weakness observed in corresponding mutant animals (7). However, we wanted to determine the cause of the balance and coordination defects in Hnf6 mutant mice. Coordination and balance deficits were quantified by rotarod and runway tests. Hnf6 mutant animals showed an increase in the fall frequency from the beam and were unable to stay on the rotarod even at low speed, indicating a severe balance and coordination deficit. To identify the origin of this abnormality, we assessed whether the development of the main CNS structure involved in the control of balance and coordination, namely the cerebellum, was affected by the absence of HNF-6. We observed that Hnf6 was expressed transiently during the first week after birth in the Purkinje cells of wild type newborn mice. We showed that, in Hnf6-/- mice, the organization of Purkinje cells became abnormal during a second phase of their development. Indeed, Purkinje cells were produced normally but part of them failed to reorganize as a regular continuous monolayer at the interface between the molecular and the granular layer of the cerebellum (Fig. 3). Thus, the Onecut factor HNF-6 contributes to the reorganization of Purkinje cells during a late phase of cerebellar development (6).

Fig. 3: HNF-6 contributes to proper reorganization of the Purkinje cells at the end of cerebellar development. In Hnf6 mutants, Purkinje cells were produced normally but part of them failed to reorganize as a regular continuous monolayer at the interface between the molecular and the granular layer of the cerebellum. Right picture shows abnormal superposition of Purkinje cells in a mutant newborn, whereas these cells were absent in other portions of the monolayer.

EQUIPMENT

- Chick embryo electroporation station
- Spinal motor neuron retrograde labeling
- Section/whole-mount fluorescence microscopy
- Cell culture facility
- Organotypic culture
- Time-lapse fluorescence imaging

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