Institutionen för Cell- och Molekylärbiologi,
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A STUDY OF THE GENERATION OF DIVERSITY IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

Developmental biology is concerned with understanding the mechanisms that govern the generation of a whole organism starting from one single cell. In the central nervous system (CNS) the development of different classes of neurons and glial cells involves both extrinsic signals and intrinsic cues that together govern the specification of different cell fates dependent on position within the CNS and the time of generation.

Different vertebrate species share many aspects of early development as well as the underlying mechanisms governing the progress of development. Therefore, a plausible assumption is that functional regions in the genome are also conserved between species. In **Paper I,** we have used a comparative genomics approach to identify Highly Conserved Non-coding Regions (HCNRs) between the human, mouse and pufferfish genomes. We find HCNRs to be statistically over represented in the proximity of transcription factors associated with spatial patterning in the developing neural tube. We show that HCNRs associated with patterning genes show an overrepresentation of binding sites for three transcription factors (Sox, Pou and Homedomain genes (SPHD)). By combining bioinformatics and large-scale expression analysis, we show that SPHD enriched HCNRs are strong predictors of CNS expression during development (83% vs. 36% of random control genes). This suggested to us that $SPHD^{+}$ HCNRs may act as CNS enhancers. Further, we isolate a putative HCNR enhancer region and show that it acts as an enhancer both *in vivo* and *in vitro*. Based on our findings, we propose a model where Sox and Pou proteins act as common activators of CNS expressed genes, while homeodomain proteins, which have been previously shown to act as repressors, act to restrict expression spatially.

While a large number of studies have provided insight into the spatial patterning mechanisms directing the generation of distinct cell types at different positions, little is known about the temporal mechanisms underlying the specification of different cell types from a common pool of progenitors in the CNS. In **Paper II,** we have addressed the question of how a seemingly homogenous population of progenitor cells in the caudal hindbrain can give rise to distinct subtypes of vagal visceral motoneurons (vMNs). We show that based on molecular marker expression we can distinguish between at least three subtypes of vMNs at early developmental time points and that each subtypes corresponds to a distinct projections pattern in the periphery. We show that these subtypes are generated sequentially and that the decision to become a specific subtype is independent of contacts with peripheral targets and cell-cell mediated interactions. Further, the homeodomain transcription factor Nkx6.1 and the orphan nuclear receptor Nurr1 are required for the specification of early born subtypes and the maturation of late born subtypes, respectively.

In **Paper III** we were concerned with the origins of oligodendrocytes in the developing spinal cord and hindbrain. Oligodendrocytes have been shown to be generated from a ventrally located domain in the spinal cord and while this ventral origin has been widely accepted, the existence of other origins remained subject to debate. We show, based on *in vitro* cultures as well as mutant analysis, that dorsal domains in the spinal cord can give rise to oligodendrocyte precursors and that these precursors have the capacity to develop to bona-fide mature oligodendrocytes based on expression of mature markers. Further we show that, at least at prenatal stages, ventrally and dorsally generated oligodendrocytes exhibit differences in expression profiles, suggesting potential differences between these populations. Additionally, our data suggests that the decrease in BMP signaling, a known inhibitor of oligodendrogenesis, in the dorsal spinal cord over time, due to the increase in the size of the neural tube, may influence the time of induction of the dorsally generated oligodendrocyte precursors in spinal cord. Also, our data from the spinal cord and the hindbrain, show that ventral oligodendrogenesis at different anteroposterior levels is governed by different genetic programs.

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