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Commentary: Engrailed 1 Shapes the Dopaminergic and Serotonergic Landscape through Proper IsO Maintenance and Function

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ABSTRACT

Two essential monoaminergic neurotransmitter systems are located within the midbrain and the hindbrain region: the mesodiencephalic dopaminergic (mdDA) neurons, which can be divided in the substantia nigra (SN), the ventral tegmental area (VTA), and the serotonergic (5-HT) neurons. In the adult brain these two types of monoaminergic neurons are critical to our neurological health. Dysfunction of the mdDA system has been associated with schizophrenia and Parkinson's Disease (PD), while dysfunction of the 5HT system has in turn been associated with psychiatric diseases such as depression and autism. The homeobox transcription factor Engrailed 1 (En1) is expressed in both types monoaminergic neurons, and is required for the correct programming and survival of the mdDA and 5HT neurons. Recently, we reported on the dual role of En1 in both neurotransmitter systems through its central role in the maintenance of the Isthmic Organizer, which is the embryonic signaling center that instructs and separates dopaminergic and serotonergic neuronal development.

Introduction

The midbrain and hindbrain are separated from each other during embryogenesis by the isthmic organizor (IsO). The location of the IsO is determined by the boundary between the expression of homeobox transcription factors Otx2 and Gbx2[1-4]. The IsO is formed at ~E7-8 of murine embryonic development and is characterized by the expression of fibroblast growth factor 8 (Fgf8)[5] and secreted glycoprotein Wnt1[6]. The IsO functions as a signaling center that pushes the development of mesodiencephalic dopaminergic neurons (mdDA) in the midbrain, and the development of serotonergic (5-HT) neurons in the hindbrain.

Engrailed 1 is a homeobox transcription factor that is highly conserved amongst species[7], and is expressed in both types of monoaminergic neurons[8-11]. Moreover, lineage trace studies showed that En1-derived cells are present in di-, mes- and metencephalon[12,13]. En1 is required for the survival of both 5HT neurons[14], and mdDA neurons: severe phenotypical changes are present in the mdDA system of the En1-homozygous mouse[15]. Moreover, animals heterozygous for En1 are considered as a model for Parkinson's Disease, as they show progressive cell loss[16-18].

En1 is Required for the Correct Function of the IsO

The authors of the discussed work continued the investigation of the En1-ablated animals[19], as they had observed additional changes in the mid- and hindbrain of En1-ablated animals

(that had been only briefly described[15]). Using an elaborate number of En1-ablated mutant mouse models (En1Cre;YFP, En1KO;Pitx3GFP/GFP and En1BatGal) the authors report that the expression patterns of mesencephalic Nurr1-targets (i.e. dopaminergic genes such as Th and Dat) are caudally extended into the hindbrain. The authors describe the presence of these ectopic dopaminergic (eDA) neurons both in embryonic and adult En1-ablated brains, and reveal that they arise at the expense of rostral 5HT neurons. Moreover, it is established that the (intrinsic) electrophysiological profile of the eDA neurons was indistinguishable from control mdDA neurons, using an En1-ablated model in which the mdDA and eDA neurons could be selectively visualized and studied, using GFP under the promotor of Pitx3. The authors continue to show that these changes are the result of aberrant expression and maintenance of the IsO, the developmental border between the mid- and hindbrain. This was visualized by aberrant and more caudally located expression patterns of important IsO-determinants such as Wnt1, Otx2, and Fgf8. Similar alterations in IsO maintenance were reported for Gbx2-ablated mice[4], as well as Wnt1- and Otx2-over-expression mice (using the En1 expression locus)[20,21]. Additionally, the authors draw a comparison to the known role of En1 in the formation of the apical ectodermal ridge (AER), which is the signaling center required for hindlimb formation[22]. Similar to the IsO, the AER is characterized by a single band of Fgf8 expression that separates the dorsal from the ventral hindlimb[23]. En1 contributes to the maintenance of this boundary, since En1-ablated animals display aberrant Fgf8 expression and an extension of the dorsal hindlimb fate into the ventral side[22,24,25].

The authors thus make a strong case for a dual, pivotal role of En1 in the mid-hindbrain region: En1's presence is required for the cell survival of both mdDA and the 5HT neurons, as well as for the correct function and maintenance of the IsO.

Strong Similarity between Midbrain and Rostral Hindbrain require Robust Division

It is easy to imagine the mid-hindbrain region as two very distinct and unique (i.e. dopaminergic and serotonergic) landscapes divided by the IsO. The opposing expression of Otx2 and Gbx2 form and underline the pivotal differences between the mid- and hindbrain[1-6]. However, these classic IsO studies also revealed the plasticity and the similarities of the midbrain and rostral hindbrain region, as slight genetic manipulations are enough to introduce ectopic dopaminergic or serotonergic neurons[19-21,26]. The hindbrain can be divided into twelve rhombomeres, and the first segment can be separated in the isthmus (R0), rostral R1 (R1r) and caudal R1 (R1c)[27]. The patterning of the hindbrain rhombomeres is beautifully orchestrated by the Hox-code: a cluster of genes that program the rostral-caudal axis, and whose location on the genome and time of expression corresponds to that rostral-caudal axis[28]. Interestingly, the Hox-code starts at the R1-R2 boundary with the expression of Hoxa2[29], leaving R1 (similar to the midbrain) devoid of Hox gene expression.

The kinship between the midbrain-R1 region is further established by the tracing studies which reveal that the cells in the mesencephalon and R1 trace back to the same En1-positive lineage[19], which was equally reported by other groups[12,13]. Moreover, a short-term re-aggregation assay illustrated that distinctly-labeled cells from chick midbrain and R1, when mixed together, are prone to remain mixed[30]. The absence of segregation behavior was mimicked by the isolated chick cells from non-adjacent rhombomeres (either odd- or even-numbered rhombomeres). In contrast, when cells from adjacent rhombomeres were mixed together, the cells would segregate[30,31]. It was shown that adjacent rhombomeres maintain cellular segregation of the different rhombomeric segments through the activity of Ca2+-dependent adhesion molecules[31]. This thus suggests that midbrain and R1 possess similar adhesive qualities, and do not intrinsically segregate from each other.

Taken together, the strong resemblance between the embryonic midbrain and R1 underlines the necessity of a signaling center that enables these two regions to each develop their own distinct monoaminergic fate. The data from the discussed work clearly establish that En1 plays a pivotal role in this process.

Future Directions and Neurological Implications

The neurogenetic similarities between the embryonic midbrain and R1, as well as their common En1-positive lineage also lead to the question: how does -during normal wild-type development- En1 promote dopaminergic differentiation in the midbrain, but repress this cell fate in R1? The authors of the discussed work raise this question, and while providing two theoretical possibilities, fail to provide molecular evidence. The authors first propose a differential interaction between En1 and members of the Pbx family, as research in drosophila has shown that presence of Exd (a Pbx orthologue) can divert the repressive effect of En1 on its targets into an activation of its targets[7]. Secondly, the authors hypothesize that En1 binds an enhancer region in the Otx2 locus to repress Otx2 expression in R1 (similar to Gbx2), but loses the competition to bind this region to Brn1/2/4 and Oct6, which are differentially expressed in the midbrain[19,32-34]. Future studies should test these hypotheses, to expand our understanding of the development of mid-hindbrain region. These data might not only contribute to our understanding of the molecular programming of mdDA and 5HT neurons, and fundamental IsO-development, but may also provide genetic leads to understand the aetiology of neurological malformations of the mid- and hindbrain in adult patients[35].

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