AN INVESTIGATION INTO THE DEVELOPMENT AND PATTERNING OF DORSAL LONGITUDINAL ASCENDING INTERNEURONS IN *DANIO RERIO*

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Dedicated to

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You left before your time was up

Contents

Abstract

The dorsal longitudinal ascending (DoLA) interneurons are an uncommon, seemingly irregularly distributed interneuron type of the developing embryonic zebrafish spinal cord. For reasons not yet understood DoLA interneurons express *tbx16*, a T-box transcription factor originally recognised for its important role in mesodermal development. This is the only cell type expressing *tbx16* in the developing spinal cord, making DoLA neurons one of the few neuronal types that can be identified by expression of a unique molecular marker.

Throughout the natural world regularity in pattern formation is frequent; mechanisms that direct the production of regular patterns have been studied and many are well understood. The creation of irregular "patterns", especially in embryo development has been subjected to far less analysis. This is largely because studies in developmental biology frequently involve methods that disrupt regular patterning while the disruption of an irregular pattern is likely to result in similarly irregular pattern. The DoLA interneurons with their unique genetic marker offer a rare opportunity to investigate the mechanisms behind irregular patterns in development. This is of particular importance in the development of the spinal cord, as most of the known vertebrate spinal interneurons appear to have irregular distributions.

The main focus of the research presented in this thesis has been to try to understand how the distribution pattern of DoLA interneurons is generated. This knowledge may then be extended to other spinal neurons and possibly to other irregular developmental patterns.

In the work described in this thesis the distribution of DoLA interneurons has been extensively examined statistically. It was found that there is an underlying cryptic organisation to their peculiar distribution. This led to the surprising discovery that these neurons migrate rostrally a significant distance along the spinal cord. These neurons were also found in larval zebrafish at much older times than has previously been described, suggesting that they may play a role in post-embryonic stages.

Notch signalling appears to have an influence on DoLA interneuron distribution since perturbing Presenilin (Psen) function affects the number of these cells. Interestingly, DoLA cell number is not affected when Psen1 function is inhibited but increases when Psen2 function is inhibited. Furthermore the wild type level of DoLA interneuron number can be partially rescued by inhibiting Psen1 function in combination with inhibition of Psen2 function.

The creation of transgenic zebrafish lines where GFP is transcribed from *tbx16* promoter sequence is described. These animals were produced to attempt to discover more about the patterning of DoLA interneurons and the function of *tbx16* during development. Serendipitously, one of these transgenic lines expresses GFP in the commissural primary ascending (CoPA) interneurons. This led to the discovery that the CoPA interneurons are marked by *mafba*/*valentino*, revealing a new unique spinal neuron molecular marker.

Declaration

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The identity and distribution of neural cells expressing the mesodermal determinant *spadetail* (Authors: Tamme, R., Wells, S., Conran, J.G., Lardelli, M.)

Independent and cooperative action of Psen2 with Psen1 in zebrafish embryos (Elsevier B.V., Amsterdam)

Cryptic organisation within an apparently irregular rostrocaudal distribution of interneurons in the embryonic zebrafish spinal cord (Elsevier B.V., Amsterdam)

Transgenic zebrafish recapitulating *tbx16* gene early developmental expression (Submitted manuscript, under review)

Signed

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