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Genetic Pathways Underlying Specification, Patterning and Connectivity of Dopaminergic Neurons in the Vertebrate Brain

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Our lab is studying the embryonic development of dopaminergic (DA) neurons in the zebrafish brain. These neurons are involved in a variety of brain activities including control of movement, neuro-endocrine and cognitive functions. DA neurons are implicated in several human neurological and psychiatric disorders. For example, a reduction in number of DA neurons is characteristic of Parkinson's disease (PD) and their abnormal stimulation is associated with drug addiction. During the process of neural specification distinct group of neural progenitors become committed towards the DA lineage, produce the neurotransmitter dopamine, assume their spatial distribution and establish brain connectivity. However, there is little known about the molecules that instruct immature DA neurons to survive and communicate with specific brain areas. Elucidating these factors has become especially important given that a proposed approach for treating Parkinson's relies upon cell replacement techniques, wherein the missing DA cells in PD patients will be supplied by transplanting DA progenitors, or stem cells. Furthermore, animal models of drug addiction have indicated that some addictive drugs cause alteration in DA neural activity and connectivity and that these changes are often mediated by factors that normally affect DA specification during development.

Zebrafish are suitable vertebrate model to investigate how DA neurons assume and maintain their identity (Fig.1). We have undertaken a genetic approach to study zebrafish mutants that display altered DA phenotypes. For example, the zebrafish mutant *too few (tof)* fails to develop hypothalamic DA and serotonergic (5HT) neurons. The observed phenotype is highly specific since other groups of DA and 5HT as well as noradrenergic neurons were not affected in *tof*. Using a positional cloning approach, we have identified the *tof* zebrafish

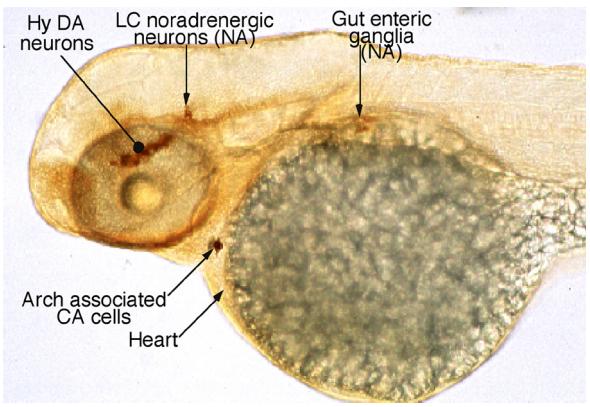

Fig. 1 *An example of zebrafish embryo stained with an antibody directed against tyrosine hydroxylase. The antibody detects the following groups of neurons: hypothalamic dopaminergic (Hy DA), locus coeruleus (LC) noradrenergic (NA), as well as arch asscociated (AAC) and gut sympathetic-neurons.*

mutant gene, which encodes a forebrain specific zinc finger transcription repressor homologous to the mammalian *fezl*. We revealed that *tof/fezl* controls the development of DA neurons by regulating the secretion of an unknown telencephalic signal, which emanate from the telencephalon (Fig. 2).

One research direction is to elucidate the unknown physiological signal, which is impaired in *tof*. Therefore, we employ combination of biochemical, functional genomics and genetic methods to seek for genes that are regulated by Fezl (termed 'Fezl target genes'). A parallel effort is made to gain information about genes function and gene order in the *tof/fezl* pathway. This is done by micro-array analysis of *tof*-mediated alteration in gene transcription in Fezl-expressing cells, which are isolated from wild type and *tof* embryos.

At the cellular level, the interactions between DA neurons and other brain cells are crucial for proper development, maintenance and

functionality of these neurons. Moreover, a major part of DA neurogenesis is the formation of axonal trajectories and synapses. To study these aspects of development, we exploit the fact that zebrafish embryos are transparent and develop externally. Thus transgenic lines that express fluorescent reporter proteins in DA cell bodies and axons are being generated. These reporter lines allow us to track DA migratory pathways and to monitor the formation of DA neural circuits during development. Using this methodology we aim to expand our earlier efforts to uncover physiological signals that control different aspects of DA specification by embarking on a comprehensive mutagenesis-based genetic screen for abnormal DA phenotypes.

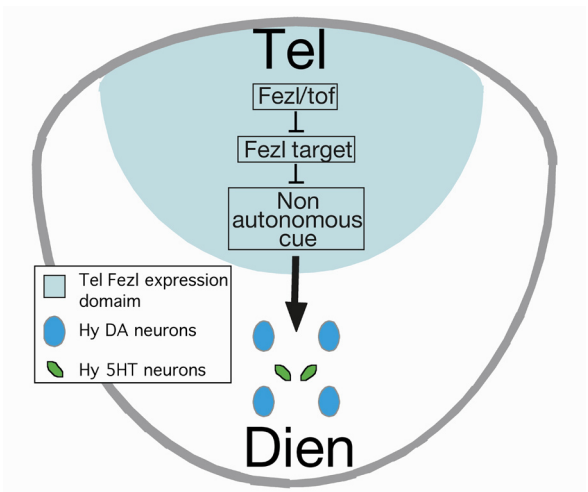

Fig. 2 *Suggested model for the development of DA and 5HT neurons in the zebrafish forebrain.*

Selected Publications

Levkowitz, G., Jorg Z., Sirotkin H. I., French D., Schilbach S., Hashimoto H., Hibi M., Talbot W. S., and Rosenthal A. (2003) *too few* is a zinc finger protein controlling the development of monoaminergic neurons. Nature Neuroscience 6, 28-33.

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