Publishable summary

of the FP7 mdDANeurodev (222999) project
'Molecular coding and subset specification of dopamine neurons generating the meso-limbic and nigro-stratal system'

Our project is aimed at identifying molecular and physiological mechanisms of the development and maintenance of mesodiencephalic dopaminergic (mdDA) neurons. The main results of the three research work packages achieved so far are:

WP1) Ventricular zone programming and early specification of the mdDA neuronal phenotype.
We were very successful in the analysis of the LEF/TCF signaling pathway and have identified cross talk to the Pitx3 pathway. Our data strongly suggest that Lef1 is not the critical nuclear effector of Wnt/B-catenin signaling in the murine VM, and that still an unknown nuclear effectors (TF's) of this pathway might function in mdDA neuron development. Further examination on the effect of B-catenin signaling has shown Lmx1a is an important target in mdDA neurons of this signaling. Moreover, we have completed the analysis of signaling pathway component expression in the proximity of dopamine neurons for the following pathways in zebrafish: WNT, SHH, TGF/Nodal, and Delta/Notch at the systems-biology level and molecular networks of DA development and function.

WP2) Mechanisms of migration and guidance of mdDA neurons.
Guidance analysis on early outgrowth, trajectory and targeting of different classes of mdDA axons has been performed and identified critical factors for this process as Frizzled3, Wnt5a and Wnt7b.
Migration analysis indicate that mdDA neurons change their mode of migration from radial to tangential as they adopt their final position and involve pathways that are regulated by Cxcl12/Cxcr4. In contrast, Slit/Robo signaling is only required for the guidance of mdDA axons.
We investigated the role of EN1 in translation regulation and identified En1/2 interactors through pull-down analysis as: factors involved in mRNA translation or mRNA transport and stability. Furthermore, experiments showed that the En1K313E mutant (unable to interact with eIF4E) is unable to up-regulate the translation of selected En1 targets and this mutant is unable to induce growth cone collapse.

WP3) Genetic programming, terminal differentiation and maintenance.
Within this part we have acquired the downstream targets of the critically important homeodomain transcription factor Pitx3 and identified the expression profile of some of these targets. The analysis have identified a Retinoic Acid (RA) dependent and independent pathway of target gene activation downstream of Pitx3. Some of these genes are critical TF's themselves as En1.
We have found that in the generation of dopamine neurons, Lmx1a and Lmx1b have both unique and redundant functions as in the progenitor domain possible through the regulation of Phox2a.
Finally, we discovered that Otx2 is the first transcription factor, with a proven role in mdDA neuron development, expressed selectively in a large fraction of VTA neurons.
Through analysis of Otx2 deleter and overexpression in mouse mutants during development we concluded that in the absence of Otx2, VTA progenitors undergoing post-mitotic transition and maturation are fated to generate a specific subpopulation of VTA neurons. Otx2 is a post-mitotic selector of the VTA neuronal subtype identity and may confer resistance to the MPTP neurotoxin. Interestingly, Otx2 function in the floor plate is distance dependent to the isthmic area, suggesting a interplay with fgf8 signaling.

The results and publications are in line with the expected output of the consortium and form the basis of a clear description of the mdDA neuronal population. These data will enable novel understanding and the design of drugs that might be applicable in dopamine neurodegenerative diseases like Parkinson's.