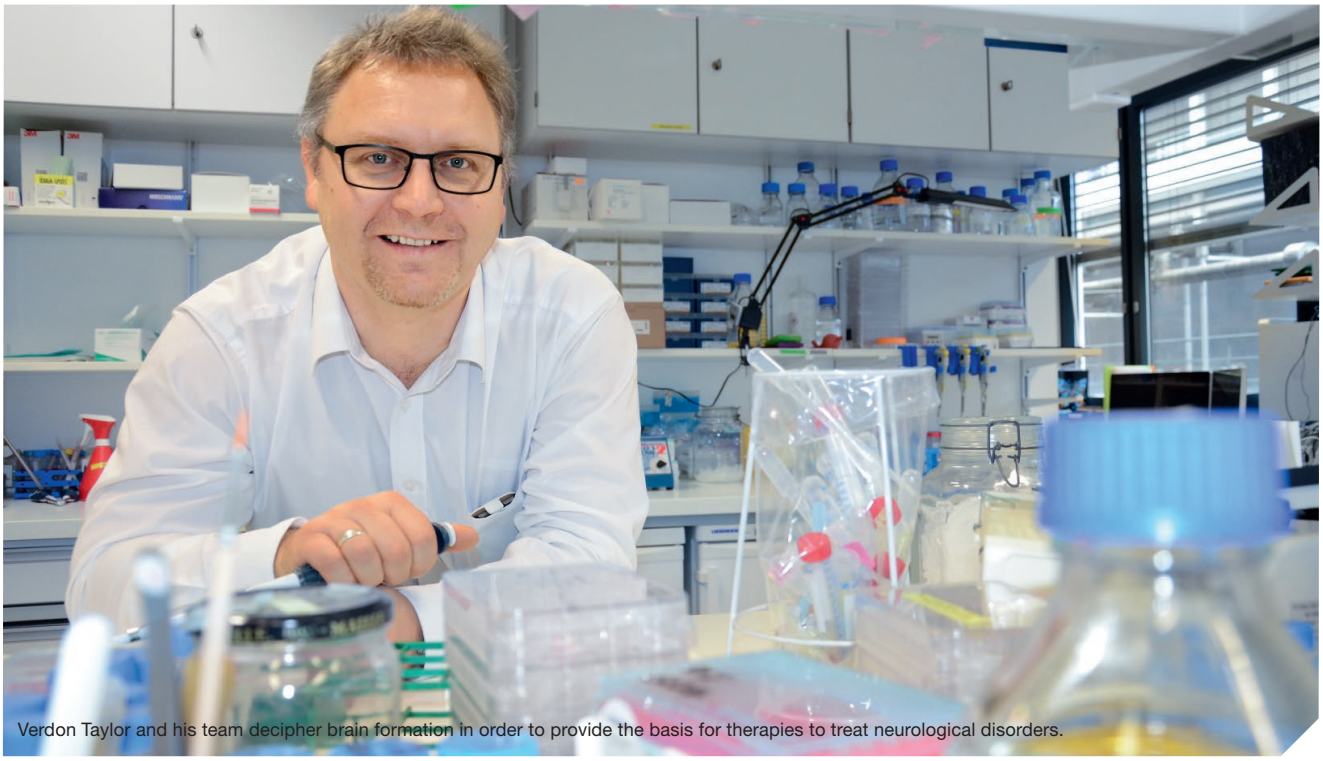
Systems biology of mammalian forebrain development (NeuroStemX)

The key to neuron production

Brain development is guided by numerous cellular processes that determine exactly when and what types of neurons arise from neural stem cells. The RTD Project NeuroStemX is bringing these processes to light and will make its findings available in the form of a complex data set.



Verdon Taylor and his team decipher brain formation in order to provide the basis for therapies to treat neurological disorders.

When Verdon Taylor and his team publish their results, there will be no single finding, like a gold nugget, which they can hold up and say, “That’s it! We’ve found it!” The truth is much better and far-reaching. They will present an entire goldmine and say, “It is stuffed full of treasures and anyone can help themselves.”

Their goldmine is a huge data set. Several terabytes in size, it incorporates billions of RNA sequences. How the genetic code manifests itself, that is, which cell types arise from a particular stem cell, is determined at the level of RNAs and the proteins they encode. The data set potentially contains all of the RNA sequences that control the development of the cerebral cortex, providing valuable insight into these processes.

Little knowledge, fewer therapies

There has never been such a comprehensive overview. Verdon Taylor, project leader of NeuroStemX, is convinced of the significance of the data for the scientific community. “Whoever detects the right pattern in the data,” he says, “will find out which RNAs and proteins control the interactions within and also between cells, so that the right neurons form at the right point in time.” This could be the key to selectively stimulating the production of certain neurons, or even producing them in the lab.

But why is this so important? It’s best to begin with the cerebral cortex. Unbelievably powerful, it is responsible for the higher brain functions of all mammals. Thanks to the cortex, human beings are able to think, move, remember. However, it is also an exquisitely sensitive structure, and insufficient growth or the loss of certain neurons can lead to disorders including autism, Alzheimer’s and some types of epilepsy.

To date, there have been very few therapies available to tackle these developmental and neurodegenerative disorders, not least due to our limited knowledge of why and how certain neurons arise from stem cells at different points in time. How are the specific genes activated or deactivated? Taylor and his team would like to address these questions.

Simple development into a complex organ

It remains extremely time-consuming to study the roles of proteins and RNAs in the regulation of gene activation and cell fate, not to mention the complex networks involving gene expression across different cell types. “Only the most recent advances in the fields of next-generation RNA sequencing and bioinformatics allow us to specifically search for complex gene networks on a more global scale,” says Taylor.

Just how do the researchers identify the critical genes in brain development? Firstly, they take advantage of the fact that the development of the cerebral cortex follows a relatively simple process. In both mice and humans, its six layers of neurons form in

the embryo in a clear, sequential manner, one after the other. In the first step, neural stem cells produce a population of progenitor cells, which generate all neurons in layer 6, the deepest layer in the cerebral cortex. When layer 6 is complete, the neural stem cells start producing progenitor cells which go on to form the neurons in layer 5, then layer 4, and so on. This process continues, layer upon layer, until all six layers have formed.

During the formation of each of the six layers, it seems that different genes are activated and expressed for each class of cell, from the neural stem cells to progenitor cells and differentiated neurons. The different gene expression patterns seen on the RNA level control the fate of the individual cells, but also govern communication between cells and establish feedback loops to regulate the next waves of gene expression.

Correctly sorted, the data reveals its relationships

"We want to find out which genes, expressed by different types of cells at different stages of cortex formation, are connected during development," explains Taylor. "This means we have to sort the genes important for the formation of each cortical layer according to cell type." In order to achieve this, the NeuroStemX team has been studying different genetically modified mouse lines. One of these mouse lines expresses green fluorescent protein exclusively in neural stem cells, another only in its progenitor cells, and yet another in the newly formed differentiated neurons.

Since the order of events in the formation of the mouse embryo's cerebral cortex is known, it was straightforward to take samples at each stage of cortical development in each mouse line. By means of fluorescence-activated cell sorting (FACS), the researchers then isolated the neural stem cells, progenitor cells and differentiated neurons and analyzed their transcriptomes using high-throughput next-generation RNA sequencing.

"We were able to carry out the mouse work up to and including the cell sorting with two cell biology research groups here in the Department of Biomedicine of the University of Basel," says Taylor. However, for the next steps, collaboration with other research groups was of crucial importance. The sequencing was undertaken by the Genomics Facility Basel. This facility develops the state-of-the-art next-generation sequencing technology that makes such extensive analysis possible.

Treasure hunting is tough work

Lastly, two bioinformatics groups at the Biozentrum of the University of Basel and the Department of Biosystems Science and Engineering (D-BSSE) of the ETH Zurich are currently analyzing the sequence data. They are looking for clues as to which signaling pathways and transcriptional networks interact with one another and in what ways so that exactly the right neurons develop at every point during cortex development. "We have already uncovered several promising networks," says Taylor, "but whether or not these will prove helpful in the endeavor to produce particular neurons, we will have to clarify in each separate case."

This is extremely time-consuming work, as possible pathways must be subsequently verified *in vitro* or *in vivo*. "Here, with a group at the D-BSSE, we are using a microfluidics approach to test the signaling pathways previously identified in culture, analyzing the cell responses at the single-cell level in real time," says Taylor. The next step will involve studying the formation of neurons from induced pluripotent human stem cells with a view to medical applications.

Although Taylor's team is only able to pursue a few of the clues, by publishing all of the data, the researchers are making their goldmine available to other scientists. The potential for advances in the understanding of brain development is huge. However, there is much work to be done by anyone who wishes to take their share of the treasure.

NeuroStemX at a glance

Principal investigator: Prof. Verdon Taylor

Research groups:

- Prof. Verdon Taylor, Department of Biomedicine, University of Basel – Embryology and stem cell biology
- Prof. Dagmar Iber, Department of Biosystems Science and Engineering, ETH Zurich – Computational biology
- Prof. Erik van Nimwegen, Biozentrum, University of Basel – Analysis of genome-wide regulatory networks
- Prof. Suzana Atanasoski, Department of Biomedicine, University of Basel – Developmental neurobiology
- Prof. Savas Tay, Department of Biosystems Science and Engineering, ETH Zurich – Microfluidic single-cell analysis of signaling dynamics
- Dr. Christian Beisel, Genomics Facility Basel, Department of Biosystems Science and Engineering, ETH Zurich – Next-generation sequencing

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