

A grain of brain

Skin cells, liver cells, neural cells – the human body is made up of various different cell types. **Hans Schöler** and his team at the **Max Planck Institute for Molecular Biomedicine** in Muenster have successfully turned these specialists back into generalists that are capable of cell division. These are able to produce different types of cells, and to develop into organ-like structures, for example into so-called brain organoids. The scientists use these to study basic processes in the human brain and the formation of diseases such as Parkinson's.

TEXT HARALD RÖSCH

When Hans Schöler began to examine the cells in early embryos back in the 1980s, he could never have guessed where his research would take him one day. His journey started with his discovery of a gene called Oct4 during his work at the Max Planck Institute for Biophysical Chemistry in Goettingen. Thirty years later, this would enable him to grow and examine different parts of the human brain.

Until quite recently, most of Schöler's peers were convinced that cells could

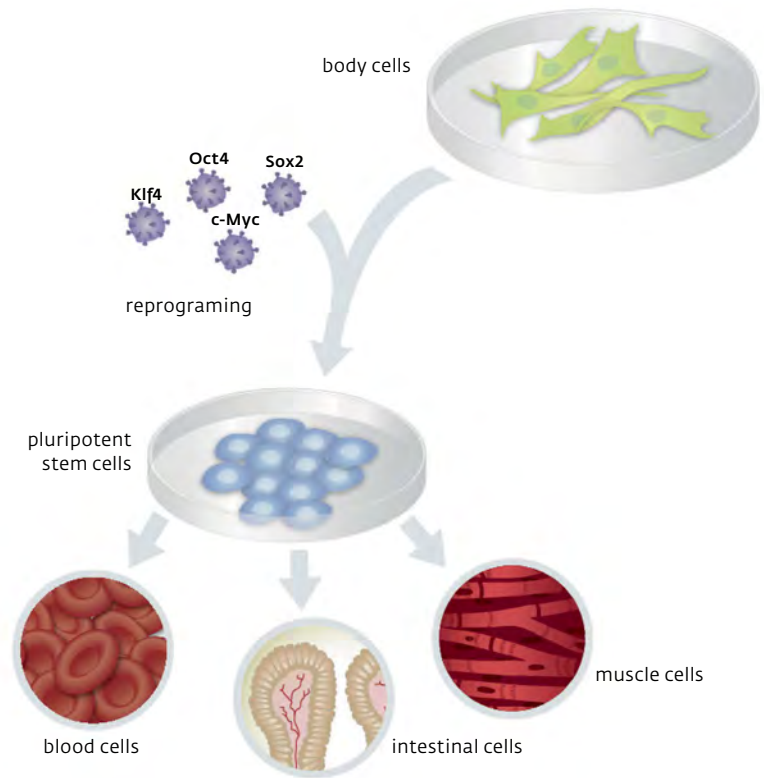
only develop in one direction: from generalists, known as pluripotent ("all-rounder") stem cells to mature specialists that are optimized for performing specific functions. Such stem cells can thus differentiate into any type of cell found in the body.

SPECIALISTS ARE TURNED BACK INTO GENERALISTS

The dogma of cell development as a "one-way street" is now history, since it is possible to use specialists to produce generalists that are by all means

able to replicate. One example of this are so-called induced pluripotent stem cells. For this transformation to be possible, the genes for the transcription factors Oct4, Sox2, Klf4 and c-Myc in particular need to be active. These genes are also activated during the early stage of embryonic development. Researchers can trigger the transformation into generalists by equipping specialized cells in a culture dish with these genes. In 2012 the Japanese stem cell researcher Shinya Yamanaka was awarded the Nobel Prize for this discovery. "When cells are transformed, their

Brain organoids in the petri dish: in the red nutrient solution, the pin-head sized neural cell clusters can be kept alive and examined over a long period of time. The darkened regions of the six month-old brain organoids are caused by pigment deposits.



From specialist to generalist to specialist: body cells can be turned back into all-rounders simply by adding viral transduction of the cells with the genes for four transcription factors. These cells are then able to develop further into any type of cell. If the transcription factor Oct4 is replaced by Brn4, the resulting cells are not all-rounders but merely multi-talented cells. These neuronal stem cells are able to generate different cell types of the nervous system.

biological clock is turned back almost to zero,” says Schöler. If it were turned back all the way, the cell could develop into an entire organism including the placenta. Cells would then be totipotent. However, this kind of reprogramming is not currently possible.

Whether a cell is a generalist or a specialist thus depends primarily on the genes that are active inside the cell. Researchers are able to control this by adding transcription or growth factors. Schöler – who is now a Director at the Max Planck Institute for Molecular Biomedicine – found out that his old friend from his time in Goettingen, the Oct4 gene, acts like a captain steering its cell vessel towards pluripotency. The other three genes in Yamanaka’s mixture could be described as the vessel’s crew: Sox2 and Klf4 can be replaced with related genes, and c-Myc is entirely expendable.

In 2012, Schöler and his team in Muenster made another important discovery: by replacing the Oct4 gene

with the related Brn4 gene, they are able to transform a mature connective tissue cell, which are part of the human skin, into a certain type of stem cell. This type of stem cell is no longer able to produce all cell types of the body, but can produce the cells of the central nervous system. Mature specialists are thus transformed back into juvenile “multi-talents”. These neural stem cells are not pluripotent but merely multipotent. “To obtain multipotent stem cells, the biological clock is not turned back quite as far as for pluripotent stem cells,” explains Schöler. Scientists refer to such cells as induced neural stem cells.

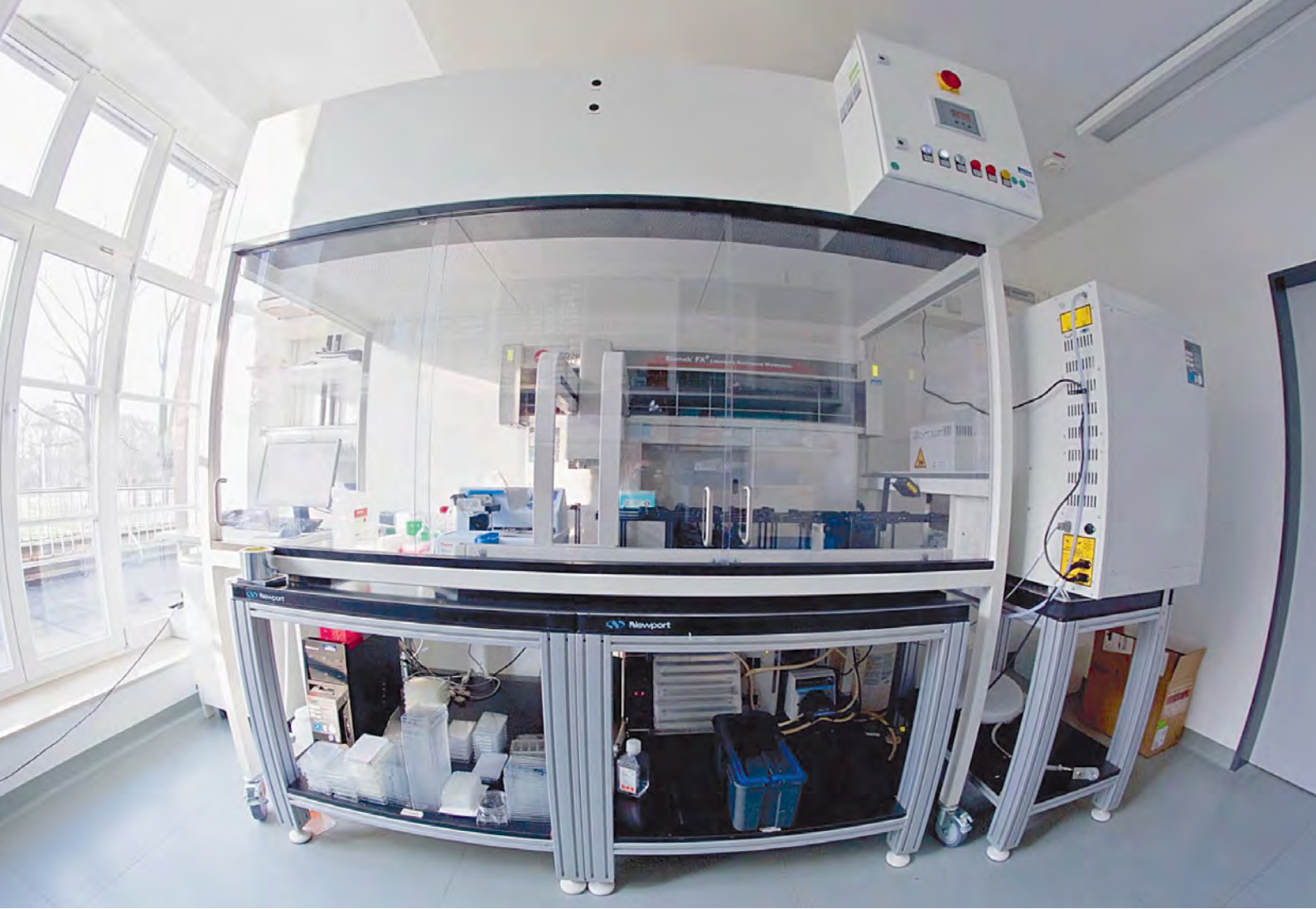
REDUCED RISK OF CANCER

The advantage of this method is that it is safer to reprogram skin cells into neural stem cells, as no pluripotent cells are produced. The risk of tumor formation is reduced as a result. However, in the future, the scientists would rather

transform mature brain cells into neural stem cells in order to produce neural precursor cells to replace degenerated neural cells.

Unlike mature cells of the central nervous system, neural stem cells are capable of cell division. Their daughter cells become the different cell types that also exist in the human brain: different types of neural cells and the far more numerous glial cells. Whereas most cells in the brain and spinal cord have lost their ability to divide, in a few areas of the brain, neural stem cells exist naturally even into adulthood. “When we introduce these reprogrammed cells into appropriate niches in brains of fully grown mice, they continue to grow and produce specialized cells that are not able to divide further,” says Schöler. This is a central requirement for medical use, as tumors, whose growth is almost unlimited, might otherwise form in the brain.

If this approach also works for humans, the method could be used to re-



The generation of brain organoids is still manual work, which makes the process unnecessarily lengthy and error-prone. The scientists from Muenster are therefore developing a robot system in order to generate large numbers of organoids in a standardized manner. This system will also be used for drug screening on brain tissues. In the future, this work may give rise to personalized therapies for all patients. The robot enables the scientists to generate, care for and test up to 20,000 brain organoids a day. By way of comparison: no more than a few hundred organoids can be cared for by hand each day. These also vary greatly and are therefore unsuitable for drug development.

place brain cells that were lost due to an illness or an accident. Induced neural stem cells would, however, need to be injected directly into the patient's affected brain areas – a difficult and unpleasant procedure. This could be avoided if scientists were able to reprogram cells that already exist in the brain into multi-talents. For example, this could be achieved with the help of RNA or other molecules that would activate genes which are responsible for transforming cells into multi-talents.

This is what the scientists are currently working on. They cultivate human skin cells in culture dishes at a temperature of 37 degree Celsius, in nutrient solutions that contain growth factors that promote cell division. The

development into neural stem cells is then triggered by viruses that integrate a "cocktail" of genes comprising Brn4, Sox2, Klf4 and c-Myc into the cells' genome. After a short time, the bottom of the dish is covered in a layer of neural cells.

CELL CLUSTERS IN A PETRI DISH

Austrian scientists and peers from the United Kingdom developed a method that allows cells not only to form a two-dimensional cell layer, but to grow in all directions – in other words, a 3D cell culture. A protein-based gel is used to provide scaffolding for the dividing cells. In this way, pin-head sized cell

clusters are generated that have a striking resemblance with the brains of early embryos.

These small cell clusters are of great interest to Hans Schöler and his team, since like the brain, these brain organoids consist of different, interconnected regions. "We are able to watch the first developmental steps of the brain, and to examine, for example, how manipulations of the genome affect development," explains Schöler. Another advantage is that the organoids consist of human cells. The scientists therefore no longer need to resort to brain tissue from mice or rats, as has generally been the case to date. This tissue differs from human tissue in many ways. >

Top Hans Schöler with a 3D model of Oct4. He has dedicated a significant part of his research career to studying this transcription factor. He discovered that the protein plays a decisive role in the formation of stem cells (The different molecular regions are shown in blue, green and red; grey: DNA).

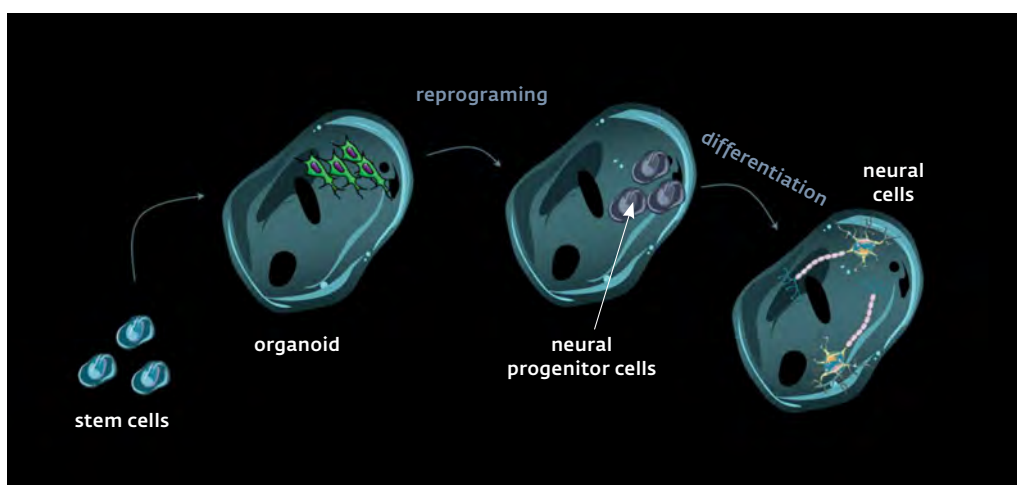
Bottom Pluripotent stem cells can specialize in the laboratory and form three-dimensional brain organoids. Scientists use these to study the reprogramming of different cell types into stem cells. These are again able to produce different types of neural cells.



The structure of the brain organoids is almost identical to that of an early fetus brain: it may for example resemble a cerebral cortex with different cell layers, into which new neural cells migrate along glial cells until they find their final position. Neural cells in brain organoids can also feature characteristics that are typical of cells in the hippocampus, a brain region that is important for learning and memory formation. Even a precursor of the retina in the eye may form.

SYNCHRONOUS ELECTRICAL ACTIVITY

Within ten to twelve weeks, the initial few thousand connective tissue cells thus develop into a cluster of brain tissue the size of a pin-head – unremarkable in appearance, yet extraordinarily complex. The cells themselves also appear to develop in the same way as they would in a natural organism. Their appearance is exactly identical to that of their natural counterparts. Electrical activity in the neural cells is also similar to that of the corresponding natural



cells. Neural cell networks that synchronize their electrical activity are formed as a result. This gives rise to wavelike activity patterns that resemble those that are made visible in EEG examinations of natural brains – albeit far less complex.

After two months, a brain organoid has reached its final size, with a diameter of up to one millimeter. “This is

the limit of what is possible without blood vessels, as oxygen and nutrients cannot sufficiently diffuse in the cell complex to supply the cells at the center,” Schöler explains. However, even if the organoids do not grow further, it is possible to keep them alive for a long time: “In our laboratory we have maintained organoids in culture for as long as a year.”

The brain organoids open up undreamt-of possibilities for scientists. They allow not only for an examination of the development of the central nervous system in the early stage, but also of the development of diseases. Schöler and his team focus in particular on neurodegenerative diseases such as Parkinson's.

DYING NEURAL CELLS

In 90 to 95 percent of cases, Parkinson's disease develops without a known genetic cause. In these cases, the disease therefore does not appear to be hereditary. The most obvious symptoms of the disease – movement disorders, stiffness, tremor and finally dementia – are due to a loss of neural cells in the *Substantia nigra*, a region in the midbrain that is responsible for controlling movement. These cells release the neurotransmitter dopamine to pass on their signals. Medication that counteracts the decrease of dopamine can alleviate the symptoms and slow down the disease's progress, but it cannot stop it.

To this date, it is largely unclear why the neural cells degenerate in this area of the brain in the first place. One of the reasons for this lack of information is that no suitable model system of Parkinson's had been available so far. "There are mouse models with mutated genes as can be found in many Parkinson's patients. Although these animals show some of the typical symptoms of the disease, there are significant aspects of the disorder that cannot be examined in rodents. The brains of mice and men just differ too much," says Schöler.

Brain organoids from human cells are expected to solve this problem. A

tiny tissue sample from Parkinson's patients is sufficient for isolating connective tissue cells. These cells feature the exact genetic alterations that cause the patient's condition. Once they have been reprogrammed into neural stem cells, they will pass on these mutations to their daughter cells. The scientists also strive to discover the mechanisms that cause the Parkinson's disease in non-inherited cases. Schöler: "The advantage compared to the previously examined mice brains is that we are able to work with human cells that develop under relatively natural conditions. This makes for a far more realistic model for studying the disease than a mouse."

A few years ago, Hans Schöler and his team used this type of genetically modified stem cells and discovered the effects of a mutation that occurs in some patients suffering from a hereditary form of the disease. To do so, the researchers took a skin biopsy containing connective tissue cells from Parkinson's patients who had a mutation in the LRRK2 gene, and reprogrammed

these into induced pluripotent stem cells. After various growth factors were added, the stem cells developed further into dopamine-releasing neural cells, like those typical for the *Substantia nigra* region that is particularly affected by Parkinson's. The scientists found that the mutated LRRK2 gene activates another enzyme called ERK in such a strong manner that this causes a misregulation of other genes and subsequently the degeneration of mature neural cells. The premature death of these cells could be prevented when the scientists rectified the mutation of the LRRK2 gene.

HELP FOR PARKINSON'S RESEARCH

Schöler and his team were able to gain important insights into the changes that occur in the cells of these Parkinson's patients. Schöler expects that in the future, even more findings will be made with the help of brain organoids. Unlike the cell cultures used in the survey, the three-dimensional brain

THINKING BRAIN ORGANOID?

Today's brain organoids are a long way from the visions shown in some science fiction movies, in which entire human brains are floating in nutrient solutions, communicating with the outside world via cables. The neural cells in the organoids are electrically active and they communicate with each other. However, they contain far fewer cells than a fully grown human brain and are therefore unable to form networks that are nearly as complex as those present in their natural counterparts. The organoids also receive hardly any signals from their environment, as they do not possess any sensory organs. This is why the scientists from Muenster prefer to refer to these brain-like structures as brainoids, rather than using the term "mini-brain" that is used in many articles about this subject.

Brain organoids at different ages: complexity that increases with age can be seen under the fluorescence microscope. While the brain organoids consist mostly of neural stem cells (green) and precursor cells (magenta) to begin with (day 10), mature neural cells (red) join the precursor cells (gray) on day 30. Mature (red) and young (gray) neural cells and glial cells (green) dominate on day 70. The nuclei are shown in blue.

organoids enable the scientists to study the effects that mutations, such as in the LRRK2 gene, have on the connectivity and functioning of neural cell networks.

LESS ANIMAL TESTING

Brain organoids from the Schöler lab may have another advantage: one day they could make many experiments redundant that are currently conducted with experimental animals. Although the brains of mice and other animals that are used as model organisms in neurosciences differ more or less strongly from human brains, there is currently no generally recognized alternative to animal experiments. Monolayer cultures of differentiated neural cells that are frequently used, are no real alternative, either. The culture conditions do not truly mimic the natural environment of the human brain. Using brain

organoids, Schöler aims to replicate the complex processes within and between neural cells, to resemble those that occur in the natural human brain as closely as possible.

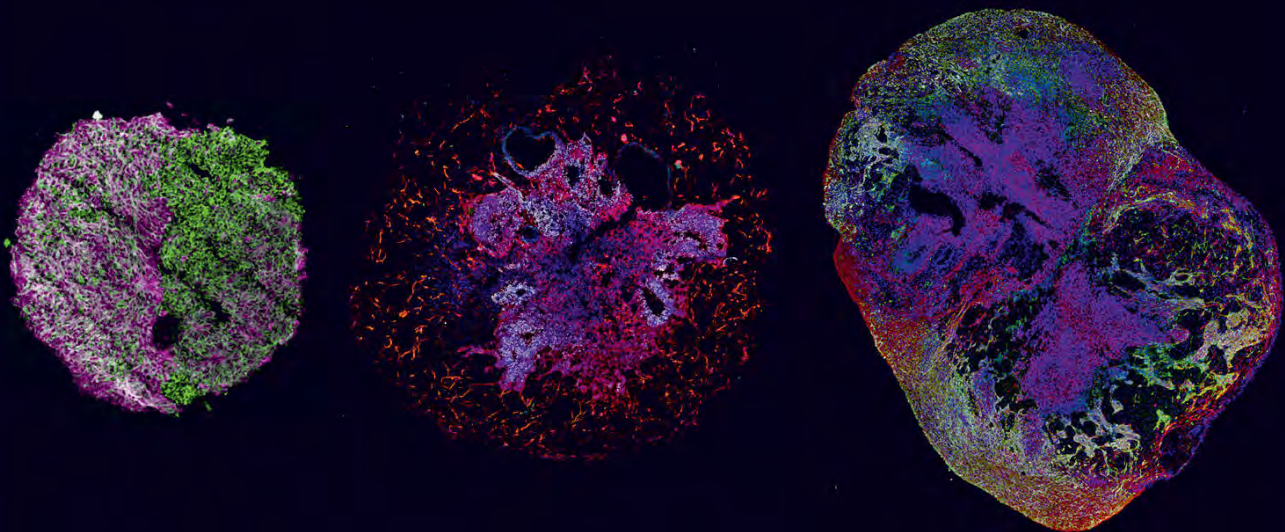
While this would not render animal experiments obsolete, brain organoids may still be a valuable alternative for many scientific questions. Schöler's work is in line with the objectives of the Max Planck Society as set out in 2017 in a general policy statement regarding animal experiments in basic research. In this statement, the Max Planck Society commits to improving the quality of research while reducing the number of animal experiments at the same time. Additional funding is therefore provided by the Max Planck Society for Schöler and his team.

To translate this vision into reality, the scientists need to meet a number of prerequisites: the brain organoids still differ too much from each other, as each

Day 10

Day 30

Day 70



» Important aspects of Parkinson's disease cannot be examined using mice, as the brains of mice and humans differ too much. Brain organoids of human cells are therefore a more realistic model for studying Parkinson's.

organoid is unique, even if generated under identical culture conditions. While under natural conditions, brain development is oriented along the body axes, cells in culture dishes do not receive this information. This is why it is difficult to predict which brain regions will be formed to which extent, making it almost impossible to achieve reproducible results. This is the main structural problem at the moment, when using three-dimensional tissue structures in culture dishes as model organs.

BRAIN ORGANOID FOR DIFFERENT BRAIN REGIONS

The first task of the scientists is therefore to improve the reproducibility of brain organoids. Their initial focus in this context is to generate organoids, which can transform into another brain region through altered reprogramming techniques. For example, they would like to produce brain organoids representing either the cerebrum, cerebellum, diencephalon, mesencephalon or myelencephalon in a reproducible quality.

Furthermore, the scientists make use of "Small Molecule Neural Precursor Cells". These are a special type of multi-talented cells that are not only capable of virtually unlimited cell division, but that can also develop into different types of neural and glial cells of the central and peripheral nervous system. One example of such cells are the dopamine releasing neural cells that exist in the *Substantia nigra*. The midbrain organoids that develop from

these precursor cells are not only far more homogenous in their structure than previous brain organoids, but can also be produced much faster and more efficiently.

Finally, the researchers from Muenster use robot systems that automatically perform and control the individual steps through to finished brain organoids. For now, the stem cells still need to be laboriously cared for by hand, and each organoid must be analyzed indi-

vidually. This process is too error-prone and too expensive for a model system. In the future, such laboratory robots will be used for drug screening on a large scale and under standardized conditions. Many animal experiments for preclinical studies will become obsolete as a result. When young Hans Schöler discovered the Oct4 gene back in his time in Goettingen, he would certainly never have dreamt that any of this would happen. ◀

SUMMARY

- Using a 'cocktail' of transcription factor genes comprising Oct4, Sox2, Klf4 and c-Myc, mature body cells can be transformed into stem cells that can produce almost all cell types that occur in the body (induced pluripotent stem cells).
- If the Oct4 gene is replaced with the related Brn4 gene, neural stem cells are produced instead of pluripotent stem cells. These are able to produce all cell types of the central nervous system.
- This approach allows for human skin cells to be reprogrammed into neural stem cells and to develop into brain-like organoids with a size of a few millimeters. Scientists can use these to perform research into diseases such as Parkinson's or Alzheimer's without the use of experimental animals.

GLOSSARY

Transcription factors: These proteins regulate (turn on and off) genes. This means that they can activate or inhibit gene expression.

Human induced pluripotent stem cells: All cells that are able to replicate by means of cell division and to develop into specialized cells are referred to as stem cells. By activating specific genes, cells in an adult that are already specialized can develop back into stem cells capable of cell division, which can produce many different cell types (pluripotency).

Neural stem cells: Unlike pluripotent stem cells, neural stem cells are "only" able to produce the different types of neural and glial cells. While they occur frequently in the embryonic brain, much smaller quantities of neural stem cells are present in the adult brain.