

One factor is enough for neural stem cells to become pluripotent. They can then be differentiated into smooth muscular cells that are found, for example, in blood and lymph vessels. The muscle cells are marked with red dye, and the cell nuclei fluoresce blue.

Potency Boost for Cells

It is one of the dogmas of biology that no specialized cell could ever change its nature and become something different. However, the researchers working with **Hans Schöler** at the **Max Planck Institute for Molecular Biomedicine** in Münster have succeeded in using a single factor from adult brain stem cells to generate the cellular jacks-of-all-trades on which regenerative medicine is pinning its hopes.

TEXT **KLAUS WILHELM**

Seen from a purely optical perspective through a microscope, the test tube revolution doesn't appear all that spectacular. However, something extraordinary recently occurred in the cells – which are identifiable as small dots – in the laboratory of the Max Planck Institute for Molecular Biomedicine. Just two weeks ago these were skin cells from a patient with Parkinson's disease – differentiated somatic cells that carry a genetic defect – and were harvested from a patient by doctors at the Dresden University of Technology. The cells were then handed over to the scientists working with Hans Schöler in Münster. This group provided the cells with a mixture of nutrients and growth factors, and also infected them with virus particles loaded with four genes bearing the cryptic abbreviations Oct4, Sox2, c-Myc and Klf4.

Over the course of about one month, these four genes transformed some of the skin cells into “human induced pluripotent stem cells,” embryonic stem cells without the embryos, so to speak. Stem cells are the cells from which all 200 cell types found in the human organism develop – cells in the skin, bones, kidneys, stomach, and so on. Moreover, scientists hope that these cells will make it possible to trace the

emergence of Parkinson's disease with a view to developing suitable drugs more easily and effectively. In this cutting-edge branch of research involving induced pluripotent stem cells, known as iPS cells for short, or “ipses” in laboratory jargon, “we are without doubt one of the world's leading research groups,” says Hans Schöler. Very few teams have succeeded in reprogramming “diseased” human cells up to now; in these cases, differentiation into disease-specific nerve cells was successfully achieved for two rare brain and muscle disorders.

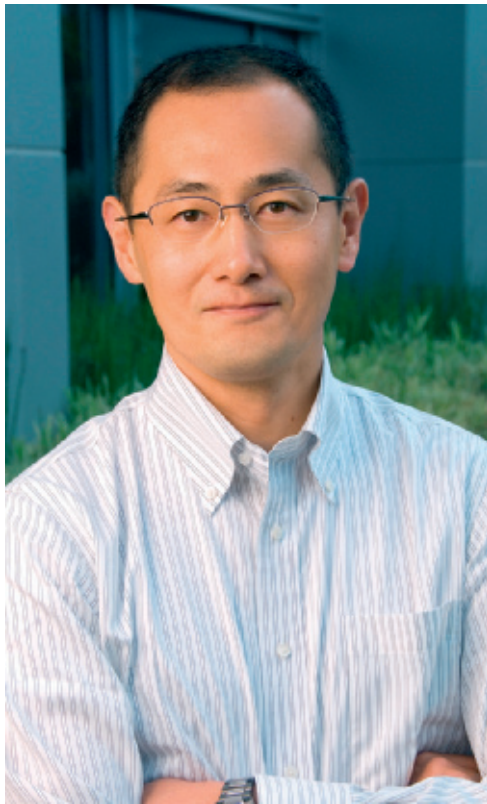
FRESH REPLACEMENT CELLS FOR DISEASED TISSUE

Up to now, visionaries could conceive of removing cells from patients suffering from cardiac infarction, diabetes, Parkinson's and many other diseases, reprogramming them into iPS cells and replacing the diseased or injured tissue with the fresh and vital cells. This would be the ideal solution and, from a purely technical perspective at least, one that no longer seems utopian. Moreover, it would also eliminate one of the main problems faced by regenerative medicine: the cells used in the treatment originate from the patient and are thus not rejected by the recipi-

ent's immune system. There is, however, one catch: from today's perspective, this kind of individual form of regenerative medicine entails enormous effort and expense – and is conceivable only with the help of automation.

As recently as 2005, Hans Schöler would not have bet a single cent that the impossible would become possible within the space of just one year, that the skin cell of a mouse would be transformed into a pluripotent stem cell by genetic manipulation alone. What this does, in effect, is turn the biological clock back. It is one of the dogmas of almost a century of modern biology that no specialized cell could ever change its nature and become something different. Once a cell has differentiated, it deactivates all of the genes in its genetic program that allow it to divide without restraint. At the same time, it switches on the genes that specialize it, making a skin cell into a skin cell.

Cells basically manufacture proteins and other molecules that they need on the instruction of the genes. In humans, only a portion of the 25,000 or so genes are switched on in every cell. Cells control the activity of their genes via complicated signaling pathways involving large numbers of proteins. This requires, above all, the services of tran-



The pioneers of stem cell research: Shinya Yamanaka from the University of Kyoto (left) succeeded for the first time in transforming mouse skin cells into cells that are very similar to embryonic stem cells. Hans Schöler from the Max Planck Institute for Molecular Biomedicine (right) managed to propagate these pluripotent jacks-of-all-trades from mouse brain cells using a single factor (Oct4).

scription factors – usually proteins that switch genes on or off. But even the transcription factors are coded by genes and are controlled, in turn, by a complex information network.

KICK-START FROM JAPAN

Shinya Yamanaka from the University of Kyoto experimented with the genes for some transcription factors in 2006. When Hans Schöler describes the pioneering achievements and the persistence of his Japanese colleague, respect shines through his every word, despite the intense competition in the field of international stem cell research. “The program of the somatic cells appeared to be so definite,” explains the Max Planck scientist, “that most researchers believed this could never work.” However, the unwavering Yamanaka provided the kick-start for what has since been unfolding “at breakneck speed” in the world’s stem cell laboratories.

With the help of viruses that acted as gene ferries of sorts, the Japanese scientist transported 24 genes for transcription factors in all conceivable combinations into skin cells. “The fact that this kind of experiment could be

carried out successfully with 24 genes was amazing in itself. I would have thought it was very unlikely to work,” says Schöler.

In a series of follow-up experiments, Yamanaka reduced the number of genes to just four: the quartet Oct4, Sox2, c-Myc and Klf4, all of which are normally switched off in skin cells. In the end, he succeeded in harvesting pluripotent stem cells. Although the process is not exactly efficient – only one in every thousand to one in every ten thousand skin cells is reprogrammed – it works! A new chapter in stem cell research began with the unlimited self-renewing iPS cells and continues to develop at an ever-increasing pace.

As far back as 1998, US scientist James Thomson succeeded in reproducing human embryonic stem cells (ES cells) in the laboratory, which marked the very first milestone. Pluripotent stem cells usually grow only at a very early stage in embryonic development: they collect in the interior of blastocysts, spherical structures that comprise between 150 and 200 cells and that form just under one week after the fertilization of an ovum and in the first eight or so cell divisions

thereafter. The pluripotent stem cells supply the growing and increasingly complex embryo with all of the different cell types it needs to grow muscles, internal organs, the brain, arms, legs, and so forth.

Back in 1998, it took enormous skill and ingenuity to propagate the extremely sensitive embryonic stem cells – as the pluripotent stem cells are known when harvested from the embryo – in the laboratory in a way that ensured they would remain unspecialized and genetically intact. Today, there are over 500 human embryonic stem cell lines and the trend is rising. However, these cellular jacks-of-all-trades also raise ethical issues, as their harvesting results in the destruction of the embryo. This was among the reasons why, in the early years of this century, German scientists in particular concentrated on adult stem cells, which can be harvested from various sources within the mature adult body. However, these cells are not pluripotent and can therefore differentiate to form only a few specific cell types.

Yamanaka’s feat was a timely one. Not only are his iPS cells pluripotent, they have also succeeded in defusing

»» Oct4 appears to command the reprogramming of cells like the captain of a ship. The other genes are the sailors.

the stem cell debate, as they can be harvested without the use of embryos. However, one major problem remained: if these iPS cells are injected into mice, many of the animals develop tumors. The reasons for this are clear. First, the viruses with the four integrated genes Oct4, Sox2, c-Myc and Klf4 slot randomly into the genotype of the mice. As a result, things like cancer genes can be activated or anti-cancer genes destroyed. Second, the increased quantity of the c-Myc gene promotes the growth of tumors. “For this reason, the Yamanaka process is out of the question for therapeutic application in humans,” explains Hans Schöler.

So the Max Planck researchers diligently searched for cells in which one or another of the four reprogramming genes is naturally active. Jeong Beom Kim and Holm Zaehres actually found adult stem cells in the brains of fully

grown mice that develop into different cell types of the central nervous system. The Sox2 and c-Myc genes are already switched on in these cells. The two cell biologists quickly demonstrated that a virus cocktail containing just Oct4 and Klf4 can reprogram these cells into iPS cells.

TRANSFORMATION REQUIRES PATIENCE

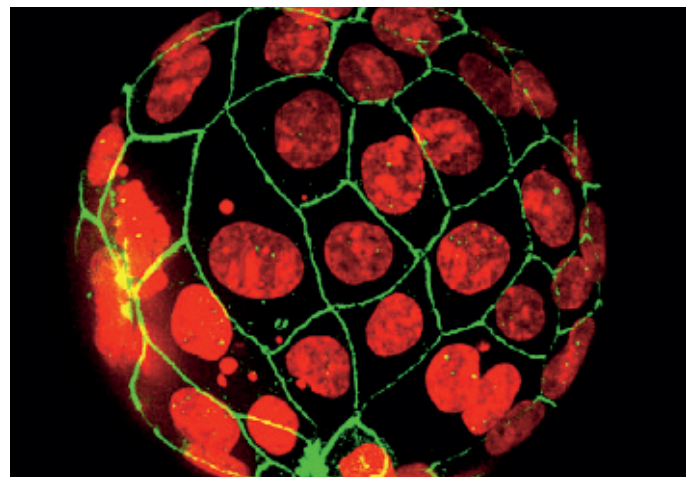
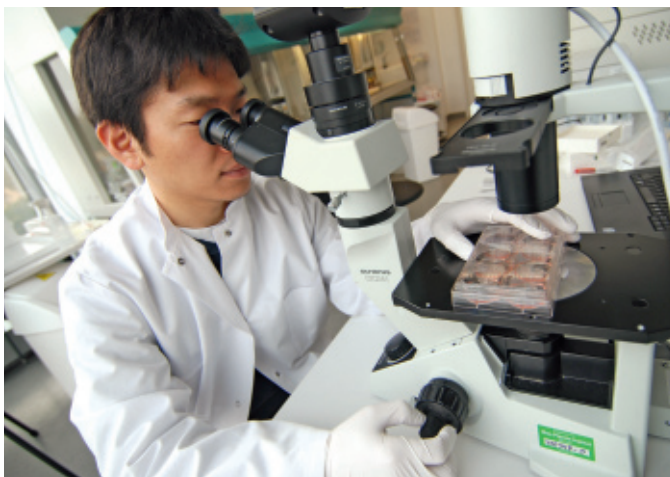
The next coup from the stem cell laboratory in Münster followed just a few months later: Oct4 alone is sufficient to grow iPS cells from adult mouse brain stem cells – as long as the process is approached with patience. If only two reprogramming genes are inserted into the cells, the transformation takes at least two weeks. If Oct4 alone is used for the cellular relaunch, three to four weeks will pass before the researchers can harvest pluripotent cells. And the

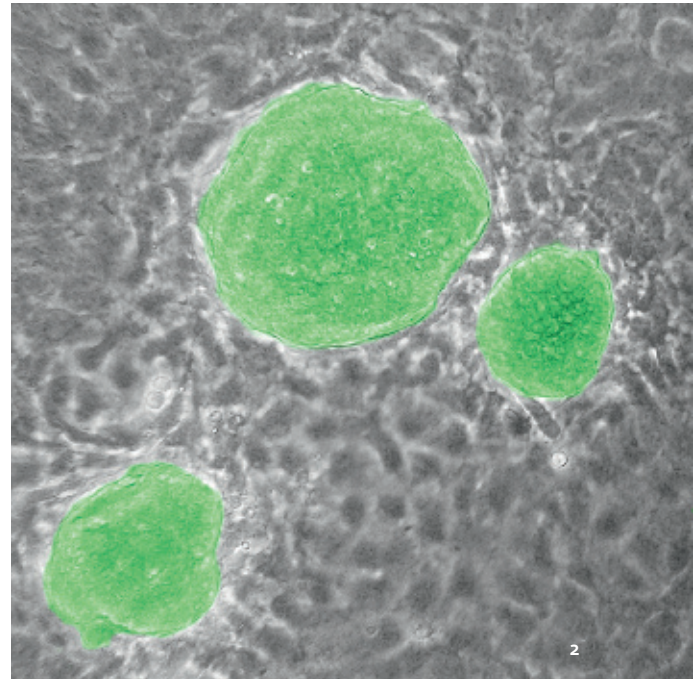
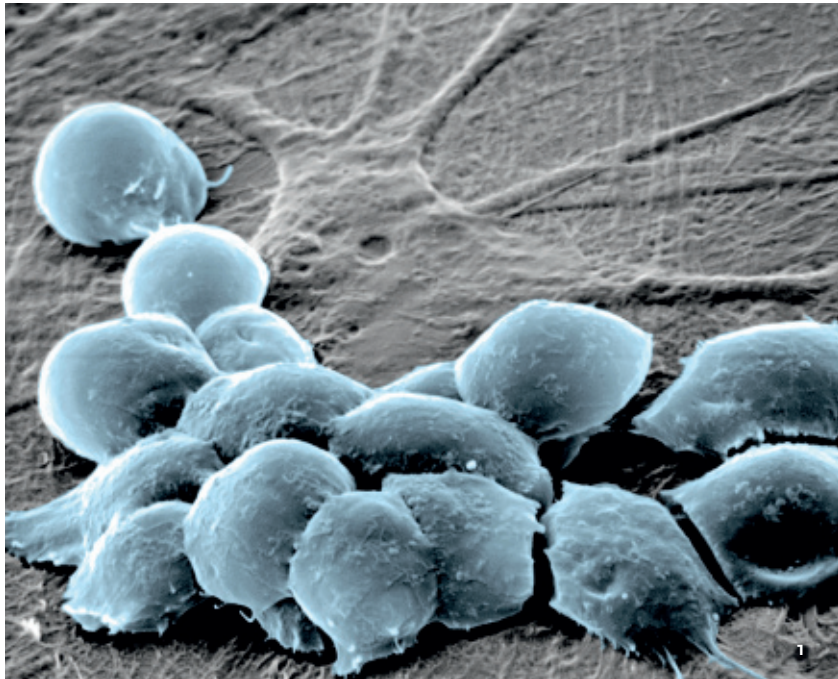
Münster-based scientists even succeeded in applying these results to stem cells from the human brain.

It is clear from this “that Oct4 appears to command the reprogramming of cells like the captain of a ship,” explains Hans Schöler. “The other genes, such as Sox2, c-Myc and Klf4, are the sailors.” Despite the fact that he has been working on this molecule and its functions for a good two decades, Schöler could previously only assume that Oct4 played such a central role in pluripotency.

Strictly speaking, the biologist was the first to discover Oct4 and related molecules in mouse egg cells at the Max Planck Institute for Biophysical Chemistry in Göttingen in the late 1980s. Over the years that followed, it emerged that Oct4 is active in all cells “that convey one generation into the next and that are thus virtually immortal,” says Schöler. According to the 56-year-old

Researcher Jeong Beom Kim (left) examines a mouse embryo at the blastocyst stage (right), which forms around five to six days after the fertilization of the ovum. The cell-cell contacts of the outer cell layer are indicated in green, the nucleus in red.





scientist, Oct4 also provides a key to the in-depth understanding of the biology of cell reprogramming. “The captain must always be on board,” says Schöler, “but the sailors can be replaced.” It appears that Oct4, Sox2 and the other genes or proteins involved regulate each other mutually. Exactly how this occurs, however, remains a mystery for the time being.

DISPENSING WITH VIRUS FERRIES

Nevertheless, the reprogramming technology is rapidly becoming more suitable for practical use. In spring 2009, scientists in California reported, in conjunction with the Max Planck team, that they had transformed cells without viruses and their genetic reprogramming load into iPS cells. Instead, the scientists transported the corresponding proteins directly into the skin cells of mice. This is no mean feat, as proteins are extremely large – on the molecular scale, at least. One particular trick proved helpful: the scientists linked a small chain of the amino acid arginine to the proteins, which had been specially produced in bacteria. This molecular “ticket” smoothes the way for their entry into the cells.

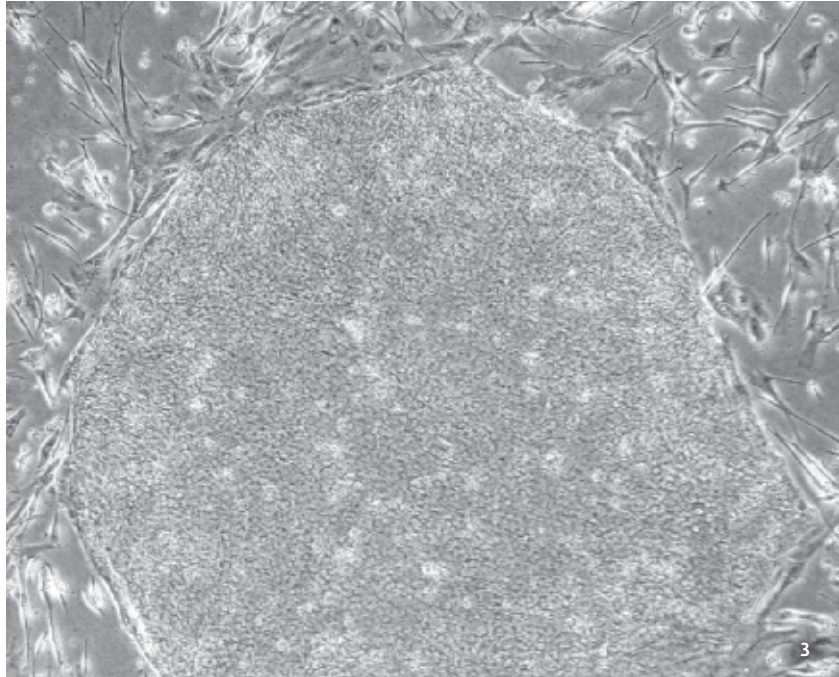
The stem cell experts channeled the cocktail of Oct4, Klf4, Sox2 and c-Myc proteins into the skin cells a total of four times. Without the repeated application, the cells would always have returned to their original state. The researchers also added a so-called small molecule – a low-dose chemical substance that helps the proteins fulfill their function – to the mix. When more than one month had passed, the researchers observed sure signs of reprogramming in some of the cells. It was thus proven for the first time that it is possible to dispense with the risky use of virus ferries. As far as we know today, the addition of the proteins does not involve any risk – not least because the proteins are broken down very quickly in the cell interior. The researchers named their new creations “piPS”: protein-induced pluripotent stem cells.

Even if large protein molecules are still required, one of the core problems of cell reprogramming appears to have been solved in terms of the therapeutic application of the method to humans (Korean researchers have already described the method for human cells). “We now have a foot in the door, but the method needs to be made signifi-

cantly more efficient,” stresses Hans Schöler. “I’m just waiting for someone to implement the reprogramming process using only small molecules,” in other words, exclusively with substances that can easily be smuggled into cells and can switch on the most important reprogramming genes, thus ensuring their pluripotent state.

“It wouldn’t surprise me if this happens soon,” says the Max Planck Director. In contrast to the transportation of genes into cells, the duration and strength of the effect of small molecules can be controlled with far greater precision: as soon as the cells are reprogrammed, the normal developmental program can unfold within them. In contrast, viruses, once smuggled in, remain in the genotype forever – with all of the corresponding risks.

In the meantime, Schöler has already sounded the next drumbeat: cells that reprogram almost automatically into pluripotent stem cells have been isolated from the testes, an organ with highly surprising peculiarities. The testes continue to produce functional sperm even into old age. But this is not the only reason why scientists suspect they may be able to find ideal source material for reprogramming there.



- 1 The research on iPS cells is developing at a rapid pace: Embryonic stem cells – in this case from a mouse – are gradually being replaced by induced pluripotent stem cells harvested from differentiated cells.
- 2 To reprogram so-called adult cells into pluripotent jacks-of-all-trades, the researchers initially needed four factors (Oct4, Sox2, Klf4, c-Myc). These stem cells were produced in this way from mouse connective tissue cells.
- 3 Because these factors can cause cancer when they are transported into cells, researchers aim to do without them, if possible. The scientists are playing it safe here: this colony of human induced pluripotent stem cells was already cultivated through the addition of Oct4 alone.

Various groups had already stumbled on cells in the testes and had stimulated transformation processes in them. For example, scientists working in Tübingen succeeded in isolating cells that are capable of transformation from human testes tissue. However, it has not

yet been established definitively whether the cells reprogrammed from this source are actually pluripotent.

The Max Planck researchers have now tracked down extremely rare germline stem cells in the testes of mice: only 2 to 3 out of 10,000 cells from the

testes are of this type. “We knew that Oct4 is switched on to a limited extent in these germline stem cells,” says Schöler, “because it also plays an important role in the formation of sperm.”

In other words, the captain of the pluripotency is actually present but is

ARE THEY REALLY PLURIPOTENT?

Researchers constantly report that they have reprogrammed somatic cells or adult stem cells into pluripotent stem cells (iPS cells). Sometimes, however, they fail to provide irrefutable proof of the pluripotency of the reprogrammed cells – even when the corresponding results have been published in prestigious scientific journals. Reliable proof of pluripotency is based on various tests:

- Proof of marker genes: Genes like Oct4, which are silent in differentiated somatic cells, are switched on in iPS cells.
- Proof of teratoma formation: If pluripotent iPS cells are injected under the skin of mice, a special form of tumor, known as a teratoma, develops. This growth contains different types of somatic cells and is similar to embryonic tumors with the formation of the three “germ layers” from which different tissue types develop.

- Proof of cell differentiation: In principle, all of the body's cell types can be produced from iPS cells in the Petri dish. Here, too, it is essential that cells from the three germ layers be cultivated and their functionality proven.
- Proof of chimera formation: Scientists inject the iPS cells into mouse embryos and prove that they are contained in the growing organism. The iPS cells are usually marked with a fluorescence gene that makes them visible under the microscope as luminous tissue. Demonstrating the presence of iPS cells that have matured into cells of the germline is viewed as particularly important in that it proves that the cells can convey their genetic information to the next generation.

The ultimate proof of the basic pluripotency of iPS cells was provided by Chinese researchers in summer 2009 based on a particular variant of chimera formation: the researchers generated viable mice from reprogrammed somatic cells that originated almost 100 percent from the iPS cells.

» We now have different systems for generating pluripotency, and they are improving all the time.

not yet fully awake and in a position to activate the sailors. The scientists roused Oct4 with the help of special cultivation conditions. They noticed something important when they cultivated the cells from the testes: when neighboring germline stem cells were a certain distance apart, they reprogrammed into pluripotent stem cells within a period of two weeks and there was no need for the introduction of proteins, foreign genes or small molecules.

“We now have different systems for generating pluripotency,” summarizes Schöler, “and they are improving all the time.” Moreover, researchers are finding more new sources of adult stem cells in the human body that can probably be reprogrammed successfully and are easy to access. For example, adult neural stem cells were recently found in the palate. It would also appear that the olfactory mucosa in the nose constitute another optimal source of these cells. This all bodes extremely well for the prospect of eventually fulfilling the dream of ethically acceptable and medically low-risk and effective regenerative medicine.

However, Schöler would first like to await the results of clinical studies carried out by doctors using “classical” embryonic stem cells. The first study of this nature is due to start soon in the US and will involve paraplegic patients. The main aim of the planned pilot study, which has not yet been approved by the US Food and Drug Administration (FDA), is to clarify the risks associated with treatment using nerve cells derived from human embryonic stem cells. “Based on the preliminary tests carried out on animals, it is unlikely that tumors will form,” says Schöler who is hopeful for the success of the study. In paraplegic animals, the inject-

ed neural derivatives of embryonic stem cells matured further at the injured locations in the spinal cord and formed healthy tissue.

UMBILICAL CORD BLOOD HELPS THE RESEARCHERS

Corresponding studies on patients based on derivatives of human embryonic stem cells could help scientists find the optimum design for future studies involving iPS cells. Ideally, this would involve the harvesting of cells from the diseased person, which could be transformed in the test tube and then differentiated as required into healthy somatic cells – such as heart muscle cells for cardiac patients or nerve cells for Parkinson’s patients.

However, due to the presumably impractical level of effort and costs associated with this approach, Hans Schöler is working with the umbilical cord blood bank in Düsseldorf, which contains thousands of samples of blood from umbilical cords. This stem cell source has two advantages. First, umbilical cord blood contains special stem cells, known as umbilical cord derived stem cells. In addition, based on a kind of immunological profile, it would be possible to establish which samples from the umbilical cord blood bank would be particularly well suited to a potential recipient. Furthermore, the genetic material contained in these cells is young and therefore contains few defects.

These are all ideal preconditions for taking samples that largely cover the immunological range of the population and reprogramming the adult stem cells they contain. The researchers would then produce precursor cells for specialized cells – ranging from skin to

heart muscle cells – from the iPS cells. These could then be implanted in patients with the corresponding disorders. “This would all take place within a clear framework,” assumes Schöler, “involving perhaps 10,000 samples for the entire German population.” Such a system would be affordable, practicable and efficient.

However, it will take many more years before this can come to pass. The Max Planck researchers have taken the first steps on this long journey: they have reprogrammed stem cells from human umbilical cord blood into iPS cells – still using the Yamanaka method. They now plan to do the same using the protein method. “We are now working intensely on this,” says Schöler. ◀

GLOSSARY

Differentiated cell

A cell that is specialized to fulfill a specific task in the body.

Induced pluripotent stem cells (iPS cells)

Stem cells that are harvested from somatic cells and that can differentiate into as many different types of somatic cells as embryonic stem cells can.

Adult stem cells

Stem cells that are also present in numerous tissues in adults. They are not pluripotent, but some can be transformed into iPS cells.

Umbilical cord blood stem cells

In late pregnancy, blood production migrates from the liver and spleen to the bone marrow through the blood of the baby. Stem cells having a particularly high reproductive capacity are thus also found in the umbilical cord blood at birth, and can differentiate into a particularly large number of somatic cells.