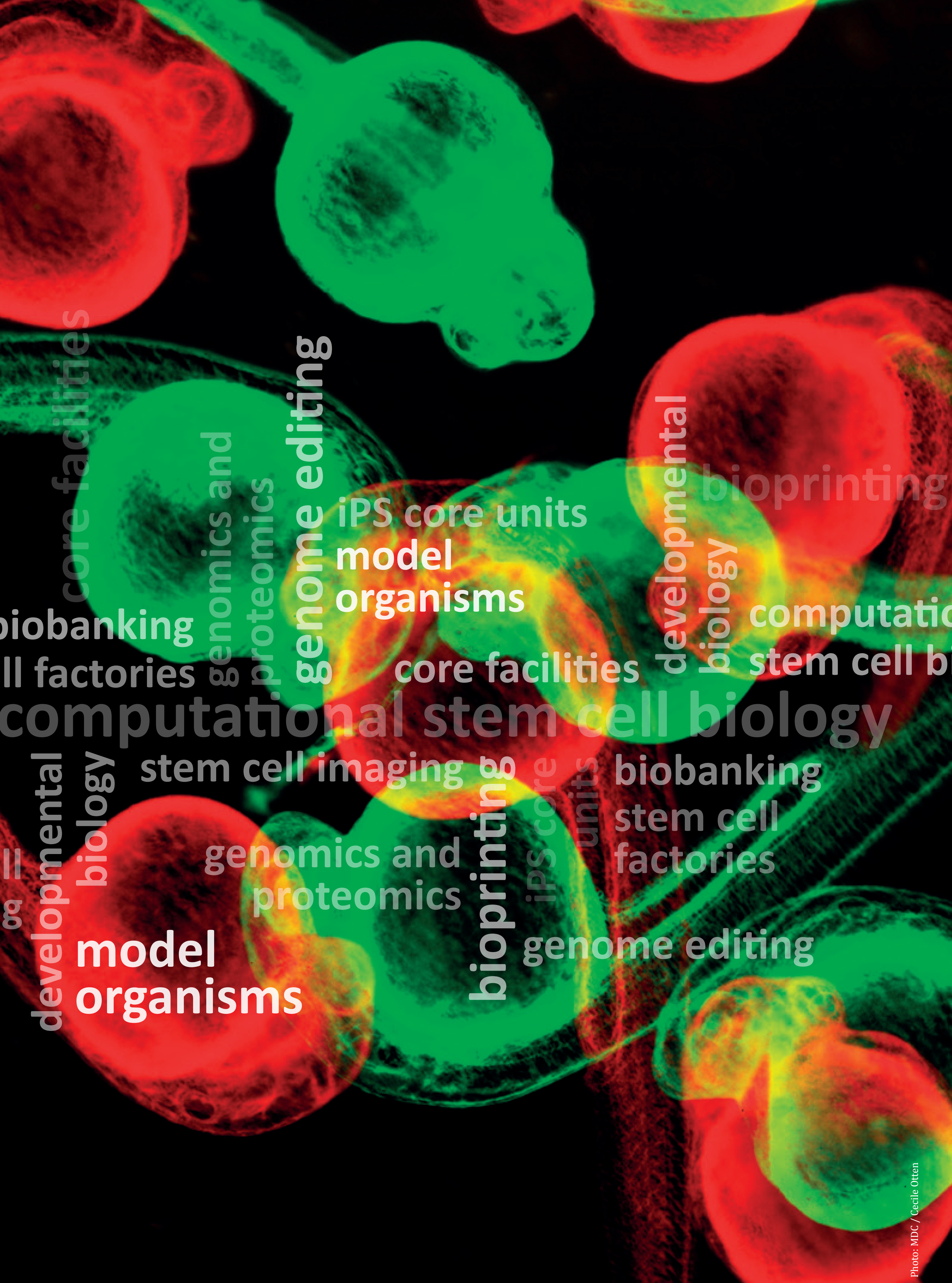


Photo: MDC / Cecile Otten

Presentation of the mobility of the embryos of zebrafish (red: before; green: afterwards)

Model organisms

Learning from masters of regeneration

Tissue stem cells are the driving forces behind growth and regeneration in humans, animals and plants. To gain an understanding of the fundamentals of stem cell biology, developmental biologists are taking a close look at model organisms with amazing self-healing abilities. Axolotl, flatworm and zebrafish are among the species that could be described as masters of regeneration. Researchers have spent years acquiring expertise on these creatures and developing infrastructures for their study. For biomedical research, mice, rats and larger animals are continuing to gain importance, but bioartificial "model organisms", or multi-organ chips, are also opening up new paths for health research.

Adult stem cells are the regeneration reserves for tissues and organs. They ensure that new cells are generated when old cells deteriorate or are injured. Developmental biologists therefore naturally focus on these cells. "There is a robust community of developmental biologists in Germany, and a number of them are interested in stem cell research," says Thomas Braun, acting president of the German Stem Cell Network (GSCN) and director of the Max Planck Institute (MPI) for Heart and Lung Research in Bad Nauheim in the state of Hesse.

Certain model organisms, both animals and plants, have proven to be ideal objects for studying regeneration processes. They can be used to study key mechanisms such as the maintenance of stem cells or phenomena like proliferation and differentiation. A whole range of organisms including flatworms, the round worm *C. elegans*, the fruit fly *Drosophila,* zebrafish, hydra, amphibians as well as *Arabidopsis thaliana* serve as models for stem cell researchers in Germany. Many resources have been set up for studying them in recent years. Complete genome information is now available even for exotic model organisms, and new molecular biological tools are opening up entirely new avenues for developmental biologists. For Thomas Braun, diversity is especially important here: "Researchers should not focus too heavily on investigating mammals. We need a sensible mix of model organisms to learn more about stem cells."

Planarians: Compact packages of stem cells

The species most favored by stem cell biologists include the planarians. These flatworms, measuring just a few centimeters in length, are easy to overlook in their natural habitats – seas and rivers. But they are truly exceptional when it comes to regeneration. Severed heads and tails grow back rapidly, and an amputated body part can even produce a complete, viable organism. This is actually not so surprising, since planarians are really just little packages of stem cells. Even the adults consist of about 30 percent neoblasts. The thing that fascinates developmental biologists is the fact that many of the neoblasts are pluripotent, meaning that a single transplanted cell can generate all the organism's cell types. But how is this pluripotency controlled and maintained? What molecular programs underlie the phenomenon?

Kerstin Bartscherer's team at the MPI for Molecular Biomedicine in Münster is taking a close look at a planarian species called *Schmidtea mediterranea.* However, planarian cells cannot be reproduced in a Petri dish. For Bartscherer, this deficit constitutes one of the system's great strengths. "For us, the planarians are a kind of *in vivo* Petri dish," she says, "meaning that pluripotent stem cells can be observed and manipulated in their natural environment." The researchers use flow cytometry to isolate the pluripotent cells and thereby determine the molecular profile of the neoblasts. In addition to gene activity studies, Bartscherer's team is using quantitative mass spectrometry to determine the protein fingerprint of the versatile flatworm cells. In this way, the Münster-based developmental biologists have already tracked down several pluripotency factors.

Zebrafish, an elegant model for geneticists

The zebrafish *(Danio rerio)* is one of the most popular model animals for foundational researchers. The animals are transparent during early embryonic development, meaning that cell structures and movements can be observed directly using a microscope. In addition, they are very well suited for genetic experiments. The zebrafish has therefore quickly become the preferred vertebrate model for studying organ development. And these fish have impressive self-healing powers. Even mature animals are able, thanks to active neural stem cells, to regenerate brain injuries. Parts of the heart muscle and fins can also grow back.

Ever since Christiane Nüsslein-Volhard, a researcher at the MPI in Tübingen and 1995 Nobel Prize laureate, brought the zebrafish to popularity as a model for developmental geneticists, scientists at many other locations in Germany have been actively furthering zebrafish research. For example, several groups of researchers at the Center for Regenerative Therapies Dresden (CRTD), a DFG research center, have focused on regeneration of the central nervous system. Didier Stainier's working group at the MPI for Heart and Lung Research is among those researching blood stem cell biology. The team recently found out that interferon gamma, a signaling molecule usually involved in inflammatory processes and infections, also plays a key role in the genesis of blood stem cells in embryos. The researchers even assume that these findings could help simplify production of blood stem cells in Petri dishes.

Using genetic screens or genetic engineering experiments, biologists in Germany and elsewhere have generated thousands of zebrafish lines. These are valuable resources that must, however, also be archived. Since 2012, the Karlsruhe Institute of Technology (KIT) has housed one such zebrafish archive. Called the European Zebrafish Resource Center (EZRC), the archive is a central facility with over 3,000 aquariums for the maintenance and distribution of zebrafish lines. It is funded by the Helmholtz Association and the Klaus Tschira Foundation. GSCN president Thomas Braun believes that central collections like the EZRC are extremely useful. "These reliable resources make our work much more efficient and much faster," he says. "They are particularly important for smaller research teams."

The axolotl, poster child of regeneration medicine

The axolotl is another vertebrate famed for its spectacular self-healing abilities. These Mexican salamanders are considered nature's best tissue engineers. If their limbs are severed or their spinal cord badly damaged, they can generate a completely functional replacement. Nerves as well as skeletal and muscle tissue all grow back perfectly. Elly Tanaka is the director of the CRTD in Dresden and a member of the extended board of the GSCN. She has been studying the axolotl's self-healing powers for many years. Tanaka and her team have already developed a number of special methods for uncovering the molecular programs behind salamander regeneration. Molecular genetic experiments in amphibians are an extremely difficult undertaking, but great progress has been made in recent years. Designer nucleases are universal molecular tools that allow genetic modifications to be carried out in a targeted way, regardless of the species (see chapter on Genome Editing, page 32). Tanaka's team in Dresden has also successfully used the CRISPR-Cas genome editing system to switch off, in a stable way, genes necessary for regeneration. Thus there now exists a powerful tool for unraveling the further secrets of the miraculously regenerating axolotl.

Zebrafish

Small rodents and large animal models

Flatworms, fish and salamanders may be unique models for researching universal mechanisms in stem cell biology, but when it comes to gaining insights relevant to human biology and medicine, mice and rats are indispensable model systems. Here as well, researchers are pinning their hopes on the new designer nucleases. Such nucleases make it possible to modify or switch off genes in a targeted way in a much shorter period of time and thereby learn about their functions. "This does make it possible to accelerate the production of knockout mice," says Braun, "but keeping the animals is still a lot of work." The regulatory burdens for animal experiments, such as the documentation requirements, have grown enormously in recent years due to new EU directives. Therefore, central resources have become all the more important here as well. The German Mouse Clinic at the premises of Helmholtz Zentrum München is an important platform, with many thousands of mouse lines available. That facility was built with support from the Federal Ministry of Education and Research (BMBF). The EU-funded European Mouse Mutant Archive (EMMA) is also housed there.

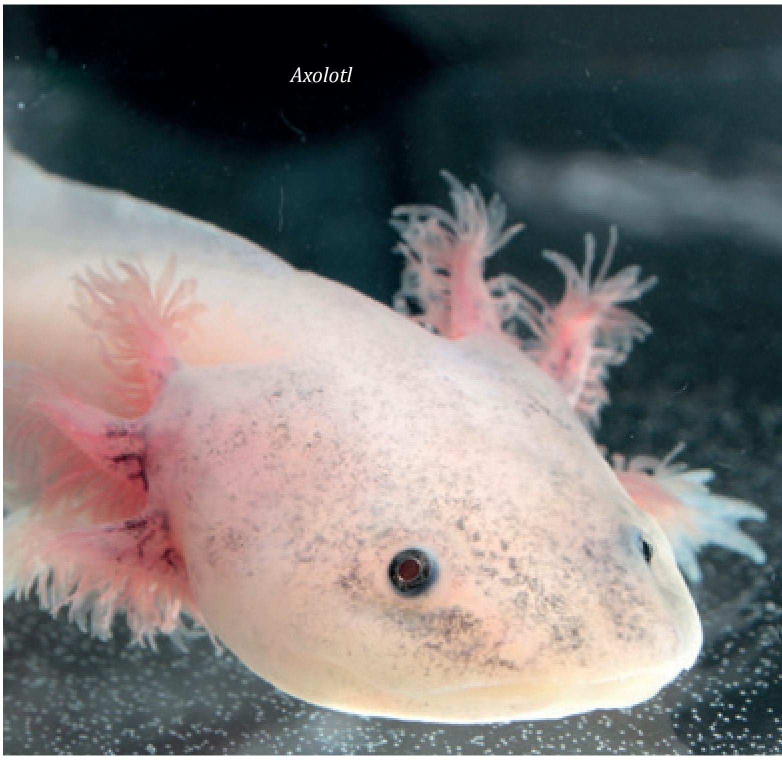
Animal models like mice and rats as well as larger animals like goats, sheep and pigs are important tools for the investigation of diseases, but in many cases there are serious limitations on how findings can be medically applied.

Photo: Fotolia / mirkorrosenau4

Multi-organ chips – humans on the specimen slide

The hope is that many animal experiments could become superfluous due to technological progress in regenerative medicine. To this end, this field of research is now producing miniature model organisms. Tissue engineers hope to shrink human tissue and organs to the size of a specimen slide so that important metabolic processes can be simulated in the lab. Pharmaceuticals could then be tested on them and clearer predictions made for the development of active substances. Researchers working with Uwe Marx and Roland Lauster at TU Berlin, funded by the GO-Bio program of the BMBF, are developing such organ chips in order to replicate important physiological processes. To do this, they are breeding organ-like structures on a microscopic scale in tiny chambers. The mini-organs consist of only a few cell types, but these already make up their own functional unit. Multiple organ systems have thus been successfully combined with one another within a very small space. The chambers of the multi-organ chip are supplied by a micro-fluid system. The researchers' long-term goal is, ideally, to pack the entire human organism onto a microchip as a modular collection of organ-like structures. A human test dummy on a chip the size of a specimen slide – that's how the bioartificial model organism of the future may look.

Text: Philipp Graf



Axolotl

Photo: Fotolia / kazakovmaksim