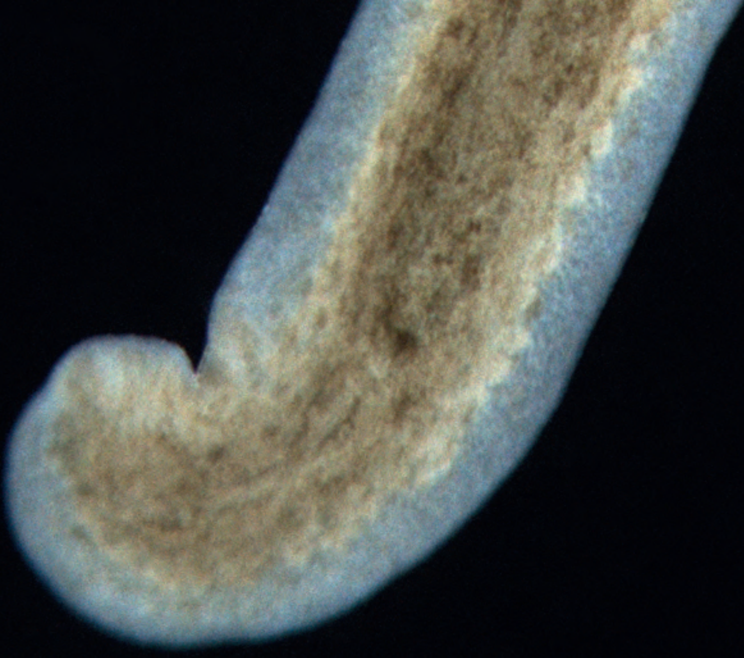


A Worm Seeks
Its Midpoint

Artists and architects of all eras have been inspired by symmetry in nature. This is hardly surprising, as symmetry is considered the epitome of beauty – and mirror symmetry is the absolute gold standard. **Jochen Rink** from the **Max Planck Institute of Molecular Cell Biology and Genetics** in Dresden is seeking to discover how organisms define the mirror plane and thereby fulfill the basic prerequisite for a symmetrical body structure. To do this he studies flatworms and their astonishing ability to regenerate missing body parts.

Planarians are almost impossible to kill – at least by chopping them into pieces. A complete worm can form from even the smallest parts; all that is required is the formation of a new body axis.

TEXT **CATARINA PIETSCHMANN**

While most of the worms sit motionless at the bottom of their culture dish, a few do leisurely rounds through their pool. With barely perceptible movements, they glide elegantly on thousands of microscopic cilia across the bottom of the plastic container they call home. This would be quite a majestic sight were it not for their large and rather comical eyes. Planarians squint and look, not ahead, but rigidly upward.

Over 60 species of these flatworms, which are all indeed very flat, inhabit a specially reserved breeding room here in Dresden. They were collected from all over the world and include some very rare eyeless specimens from caves, and others that have a string of eyes along the edge of their bodies. Flatworms are usually between 0.8 and 2.5 centimeters in length, but there are also some veritable giants that measure one meter in length. They usually come in different shades of brown or black, but some also have distinctive dots or stripes. They are easy to care for in the laboratory and pounce on the bits of calf liver or mealworms fed to them in their plastic boxes.

Given their bizarre appearance, one might almost think they made a deal with nature millions of years ago: Let others be beautiful, like the ladybirds with their red wing cases with flashy black dots. We don't need all that. Give us something else instead: eternal youth!

For the price of eternal youth, some flatworm species even forego sex. The species that reproduce asexually are practically immortal, because every tenth cell in the worm body is actually a stem cell that can generate all of the different cell types indefinitely. For this reason, planarians self-renew constantly. For every dividing stem cell, a specialized cell dies, so the shape and size of the worms remains unchanged.

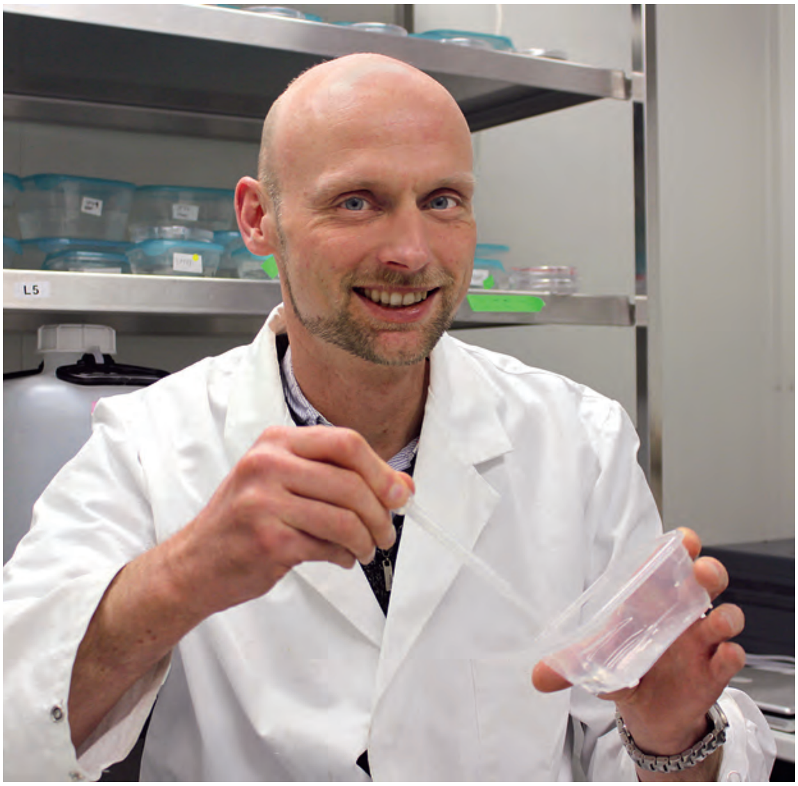
ONE BECOMES TWO

To reproduce, all they have to do is hold on tight with the tip of their tail while the head moves on. "After a few hours, the body tears apart and two complete worms regenerate from the two pieces in just two weeks," explains Jochen Rink from the Max Planck Institute in Dresden. This makes planarians one of the most sought-after test objects in stem cell research. Their extraordinary regeneration capacity may hold the key to eternal youth and to cures for many illnesses.

But there is something else about flatworms that fascinates Rink: not only can they build a complete organism from any random tissue piece, they even manage to restore perfect symmetry during regeneration – regardless of whether the original animal was divided into two pieces or twenty. For example, strips of tissue that are cut from the side of a flatworm initially curl up into a tight corkscrew spiral and develop into completely independent organisms in a matter of days. This distinguishes flatworms from other regeneration specialists, such as the axolotl: although these salamanders can regenerate missing body parts, organs and even parts of the brain, the cut-off piece never develops into a new animal, but rather perishes.

Something the two animals do have in common, though, is the symmetry of their body plans. The foundations of symmetry are already laid in the early embryonic stages. Also the process of regeneration in planarians must ensure that the resulting body is symmetrical, or that the new parts re-complete the symmetry of the existing body. "This so-called bilateral symmetry – two mirror-image body halves – is a basic structural principle of higher organisms," says Rink.

Bilateral symmetry requires, first, an axis of symmetry, known as the midline. But how does an organism actually define a line? It is already known that evolution has come up with different ways of doing this. In vertebrates, for example, the dividing cells of the early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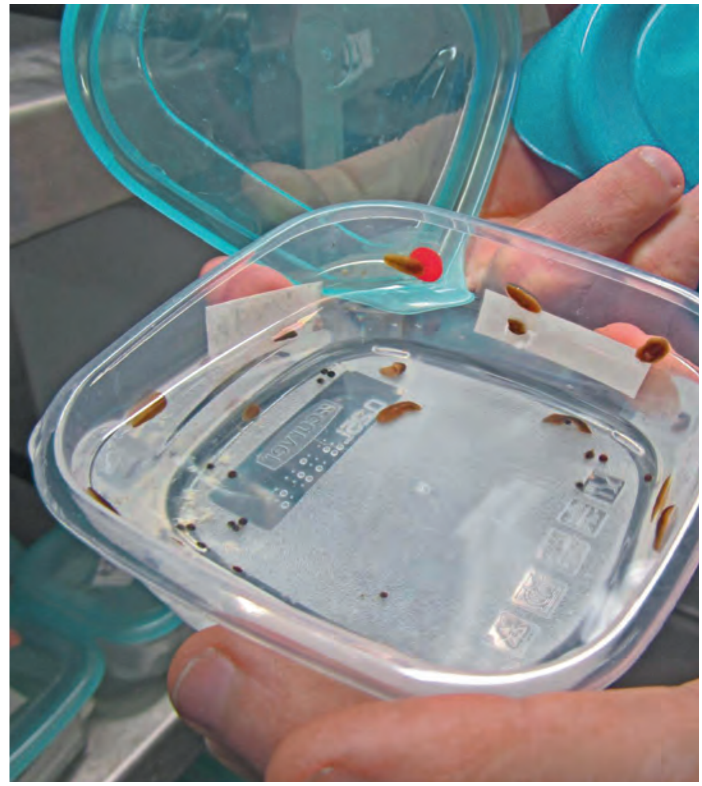
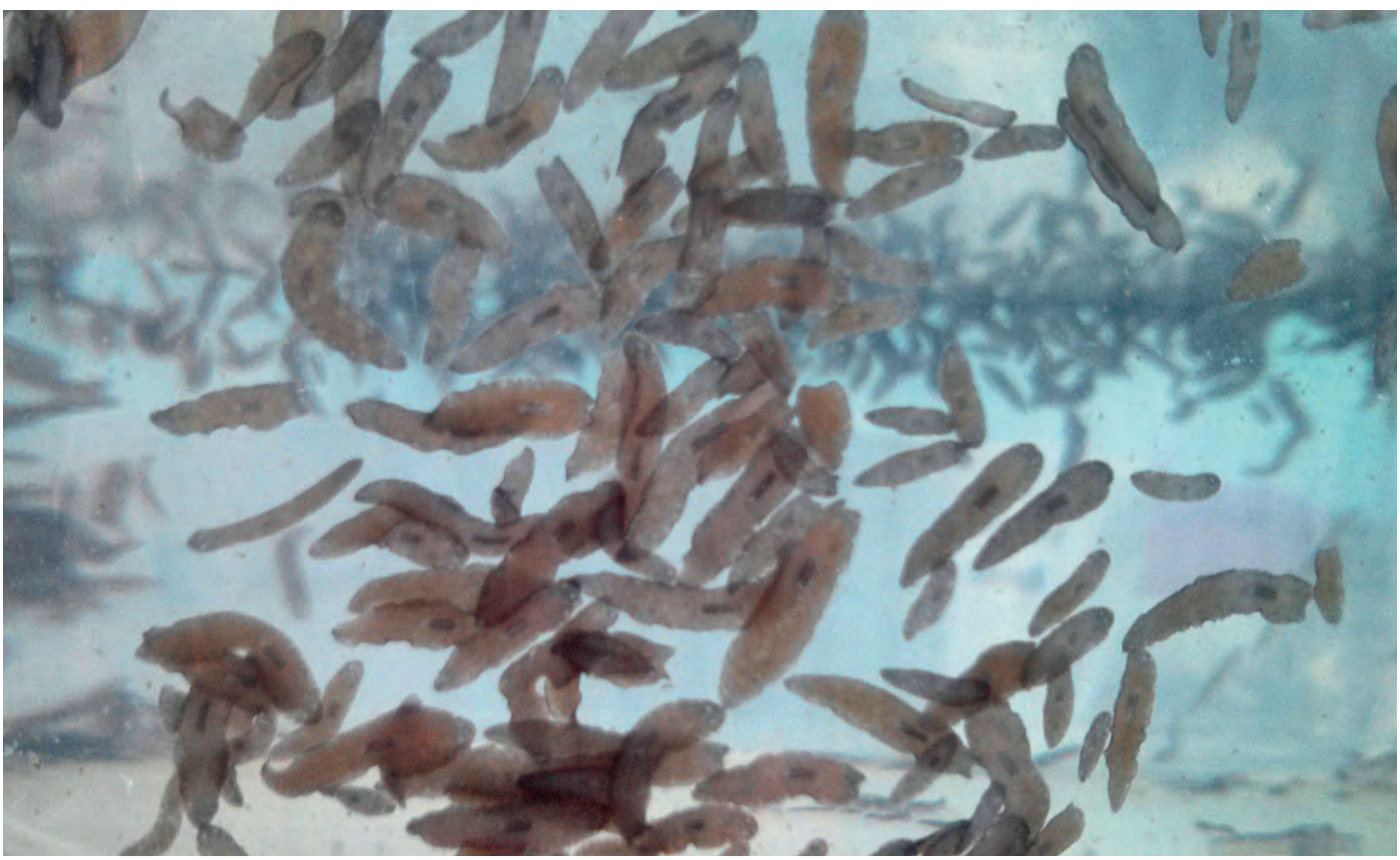




Left page Planarians are easy to care for: Apart from feeding and occasional water changes, Jochen Rink (top left) doesn't have to do much for them. They live in standard plastic household containers in the laboratory of the Max Planck Institute in Dresden (top right). They can reproduce very rapidly in these containers (bottom).

This page Planarians need the hedgehog signaling pathway to regenerate lost tissue. A middle piece (left) usually forms a head with a brain (blue) and the tail with two intestinal strands (green). If there is not enough hedgehog available (center), a normal head grows, but no tail. If there is too much hedgehog (right), a complete tail is formed instead of the head.

embryo migrate and gradually envelop the yolk mass like a tight-fitting hat. A subset of these cells reverses direction at a defined point, the so-called organizer, and marches back up against the flow on the inside of the hat. This produces a hemisphere composed of two cell layers.

STARTING POINT FOR THE MIDLINE

"The reversal point later turns into the midline of the body, and the head and tail form at its opposite ends." Signaling networks in the cells control this process by switching certain genes on and off at the right time. In short, nature proceeds thus: a point is defined from which the cells migrate in opposite directions, and voilà – the line is created.

The fruit fly *Drosophila* takes a different approach to midline formation. Its embryos aren't spherical, but rather cigar-shaped. Signaling substances in the cells define "top" and "bottom," which correspond to the back and belly halves of the future embryo. "Top" and "bottom" signals suppress each other, but because the belly signals are stronger, all that remains of the top signals in the end is a clearly defined line along the back, which again becomes part of the midline of the emerging fly body.

And what about planarians? Rink and his colleagues started by looking for genes that are active only at the midline. The genes they encountered here included the BAMBI and Slit genes, both of which are active only in cells located exactly along the midline: BAMBI is active only in a narrow strip along the back, and Slit marks a broader V-shaped strip extending all the way from the head to the tip along the animals' belly side. Thus, the midline is defined, not by a single cell type, but by multiple types. Here, too, nature probably uses the same molecular mechanisms throughout the animal kingdom, as Slit is also active along the midline in flies and humans.

In the early 1960s, biologists accidentally discovered the dramatic consequences that arise when the formation of symmetry in an organism malfunctions. Deformed lambs with only one eye were born on a few meadows in California. Like the Cyclops in Greek mythology, their eye sat in the middle of their head. It emerged that the ewes had eaten corn lilies. These plants contain a toxin that inhibits recognition of the hedgehog signaling protein in embryonic cells. Surprisingly, the defect was limited to the lambs' heads. The animals, which died soon after birth, had four normally formed legs.

"If we disrupt the hedgehog signaling pathway in planarians, something similar happens," reports Rink. He and his colleagues varied the strength of the hedgehog signal in the worm body, cut off the head and tail, and observed the regenerating pieces. If the piece had far too little of the hedgehog signal, a perfect new head developed, but the tail was severely truncated; if too much of the hedgehog signal was present, the regeneration of the tail was perfectly fine, but the pieces regenerated a tail in place of the head. And if the hedgehog signal was only slightly raised, worms

with stunted and eyeless heads or, again, one-eyed Cyclops worms, regenerated. Except that in the case of the lambs, the one-eyed lambs had too little of the hedgehog signal.

NEURAL PATHWAYS FORM A LOOP

The researchers then eagerly turned their attention to the genes that mark the midline. In the animals with stunted heads, the midline gene BAMBI was active right through to the tip of the tail, but no longer in the head region. The stunted heads were also missing the nerve tracts, which reconnected into a loop below the head area. "So these animals don't have a brain, just parts of the peripheral nervous system." And because the new head tissue lacks any midline information, it is no longer bilaterally symmetric, but rather radially symmetric, similar to a sphere or a circle. In the one-eyed worms, in contrast, the BAMBI gene activity extends all the way from the tail tip to the head, but the nerve tracts are spaced much closer together than usual.

Rink concluded that "The midline targets the head and tail as end points. If these parts of the body are fully formed, then there is a complete midline. If the head isn't fully formed, problems with the midline arise, and result in one-eyed animals." Clearly, the signaling systems that designate the midline are so finely tuned that they don't tolerate any kind of disturbance. In order to track how a new midline forms, the researchers then cut narrow strips from the sides of some worms and used a green dye to visualize the activity of the Slit gene in these strips.

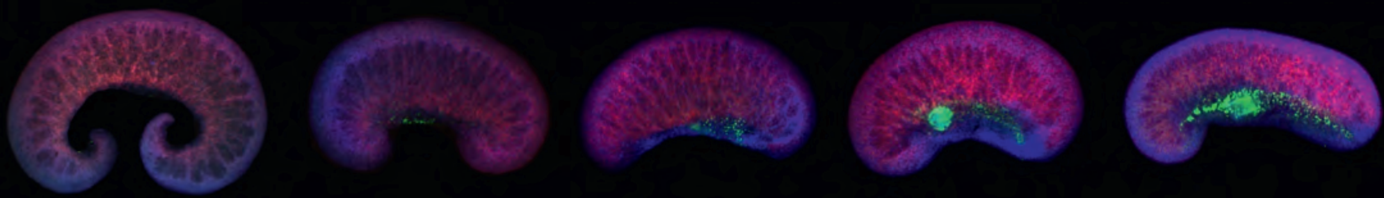
Immediately after cutting, the strip evidently contained no midline information. Just one day later, however, the first green cells appeared under the microscope. "Not in the middle, but along the edge of the wound," Rink explains.

On the second day, two apparently distinct populations of green cells had formed – one centered approximately halfway along the length of the strip, and a second one right at its front end, likely the precursors of the midline in the head. Further, some green cells had already settled beyond the new midline, which eventually developed from the wound edge. "We still don't know where these cells come from." After six days, the pair of eyes had formed. The central population of green cells expanded and became the pharynx, which sits right in the middle of the animal and serves as both the mouth and the anus. The midline doesn't extend to the tip of the tail until the digestive tract is fully formed. The cells seem to operate like a construction team that completes the work at one location before moving on to the next.

SIGNALING SUBSTANCES DETERMINE POSITIONING

But how does the strip know that it was originally located on the right-hand side of the flatworm's body and that it is now missing its left side? And what signals the completion of regeneration and the resulting growth arrest? "We still don't know exactly how these processes work. They may be controlled by the concentration of signaling substances in the body," says Rink. For example, if the concentration of a messenger substance were to decrease steadily from front to back, a cell could identify its position in the body based on the local concentration. If a cut were to be made at the location of the cell, this information could again be utilized to instruct regeneration of a head rather than a tail, or vice versa. The body and wound edges, too, likely assume important roles in the process of regeneration, as the cells from the

Photos: Max Planck Institute of Molecular Cell Biology and Genetics



Above A narrow strip of lateral tissue doesn't usually contain any midline cells. After just one day, the first midline cells (green) form – not, however, in the middle of the tissue, but at the wound edge. Over the next few days, a line forms between the old (violet) and new (blue) tissue. Through the integration of new cells above this line (blue areas), it shifts more and more toward the middle until it finally becomes the midline.

Left page Regeneration of the midline: If a narrow strip is cut off from the side of a complete animal (gray in the graphic), the strip initially curls up in a corkscrew. Two days later, the tissue unfolds again and the cut has healed. After six days, a lot of new tissue has already formed on the former edge of the strip (light brown). On the tenth day, the strip has almost entirely reformed the missing parts. After 14 days, a mirror-symmetrical worm has formed from it again.

Photos: Max Planck Institute of Molecular Cell Biology and Genetics

back and belly halves meet here. The tissue is able to recognize this. Moreover, some signals must be able to reach all cells of the body, since pricking the worm with a needle raises an alarm signal that compels all stem cells to divide more rapidly.

The system is so well controlled that it can estimate the severity of the injury. "This is important," says Jochen Rink. "The worm shouldn't grow a new head every time it suffers a small tear."

Rink's team also studied the genetic relationships among the flatworm species in the Institute's collection, as well as their capacity for regeneration. The scientists have evidence that the species that can no longer regenerate were once able to but lost the capacity over the course of evolution. "A single signaling pathway changed in them. Interestingly, this happened several times and in different locations," Rink says. He would now like to find out why this is the case.

The scientists also discovered another striking correlation: species that can no longer regenerate reproduce sexually. "So we suspect that the extraordinary regeneration capacity of planarians doesn't serve the purpose of wound healing at all, but that of reproduction." The ability to simply split in two rather than having to go through the trouble of looking for a partner is an advantage if you happen to live in a puddle that will soon dry up.

ONE INDIVIDUAL, MANY STEM CELLS

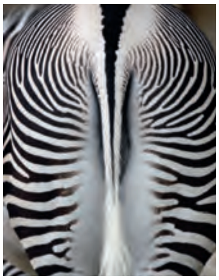
Unlike in the fruit fly and zebrafish, it is not yet possible to make genetic modifications in flatworms. The ability to insert a gene for a fluorescent protein into the worm's genome and simply observe the growth of the midline would facilitate research considerably.

The fact that this has not been possible up to now is probably due to the organism's high number of stem cells. "When stem cells constantly divide in an animal that doesn't age, and each division carries an inherent risk of mutation, the animal should consist of many stem cell lines that mutate independently of one another. So maybe the worm is no longer a single individual, but many," says Rink, developing this idea further. A collective, as it were. Together with colleagues from the Max Planck Institute in Dresden, Rink's research group recently decoded the genome of planarians. This now provides a basis for testing the collective theory. Flatworms are early descendants of the last common ancestor of all extant animal species, including humans. "This ancestor must have had a head and been bilaterally symmetrical." It would therefore appear that this form of mirror symmetry existed before the first animals developed a rigid spinal column and fins, legs or wings. The body structure of most living organisms today is mirror-symmetrical.

"Symmetry is very deeply rooted in biology. The underlying molecular programs were developed 500 million years ago in the Cambrian period," says Rink. Everything after that was merely a variation of the theme.

Bilateral symmetry, however, is more than a structural feature. It is the prerequisite for the definition of "front" and "back," and "left" or "right." Thus, the head-tail axis also dictates the direction of movement. "Maybe a bilaterally symmetric body plan became essential once organisms began to move using lateral appendages," says Rink. "If your right fin is bigger than your left one, you can only swim in circles." Unequal distributions of wings or legs are similarly inconvenient. On the other hand, most plant leaves also have a clear axis of mirror

SYMMETRY IN NATURE



Symmetry exists in both animate and inanimate nature. Sugar and amino acid molecules can have a mirror plane or behave like image and mirror image – a phenomenon known as chirality. A different spatial arrangement in an otherwise chemically identical molecule can alter its characteristics considerably: with mirror-image scent molecules, it can give rise to a grassy or citrus smell, and with drugs like thalidomide, it can result in the drug being either effective or harmful. Most minerals also arrange their atoms in symmetrically structured crystal lattices, like the double pyramids in diamonds and the cubes in table salt. Even snowflakes always have a six-fold axis of rotation.

Nature presents a special kind of symmetry in the structure of spirals. These arise in all orders of magnitude, from the DNA double helix to the arrangement of seeds in a sunflower head, animal horns, and galaxies. Because spirals don't have a mirror plane, they are per se asymmetrical. However, they can behave like image and mirror image with each other – like the right and left hand. Organisms generally show a preference for one direction of rotation: in snail shells, the right-left ratio is 20,000 to 1. The field bindweed plant, in contrast, spirals exclusively to the left.

While the mirror plane is usually the only symmetrical element found in the animal kingdom, plants frequently have a combination of mirror planes or multiple axes of rotation or rotary mirror axes.

symmetry even though they never move. The deeper reason for symmetry in biology may therefore lie in transport or growth processes.

Overall, symmetry must have a purpose. Nevertheless, it is never perfect – just good enough for said purpose. "The right arm will never have the exact same number of cells as the left one," says Rink.

So was symmetry a basis for complexity? The answer is probably yes, but there is an exception. We know of one single multicellular organism that is not symmetrical: *Trichoplax adhaerens,* which translates approximately as "hairy sticky plate." It looks like a misshapen pancake. Thanks to muscle cells and a carpet of eyelash-like hairs on its belly side, this tiny, three-millimeter-long animal can move like an amoeba. "It's a fascinating case, as it shows that symmetry isn't absolutely essential for multicellular life." Also, *Trichoplax* may have once been symmetrical and lost the trait due to a whim of nature. ◂

TO THE POINT

- **The BAMBI and Slit genes are involved in determining the middle of the body in flatworms – and possibly also in other organisms.**
- **Concentration gradients indicate to cells where they are located. This allows them to "remember" their position and orientation in the body if they become separated from the rest of the organism. Missing parts are then newly formed in the correct position.**
- **The capacity of planarians to regenerate entire body parts may not serve the purpose of wound healing, but instead represent an extreme form of asexual reproduction.**

GLOSSARY

Bilateral symmetry: This is the predominant form of symmetry in the animal kingdom. Over 95 percent of animal species are bilaterians, including humans. The middle of the body constitutes the axis of symmetry, at which every point on the right and the left can be reflected and brought into congruence with its opposite point. Some groups, such as the sea urchins and starfish, are bilateral as larvae but later become radially symmetrical.

Hedgehog: A signaling protein that controls embryonic development in most animals. The protein is part of a signaling pathway that consists of various enzymes and receptors within cells. The signaling pathway must have emerged at a very early stage in evolution, as it arises in almost all animal groups. In insects, it controls the formation of the body segments and wings. In vertebrates, the hedgehog signaling pathway is responsible for bilateral symmetry and the arrangement of limbs. Damage to this signaling pathway leads to significant malformations and can cause cancer.

Photos: shutterstock (3)