A Repairable Heart

Newts possess the almost magical ability to regenerate damaged tissue, making them unique among vertebrates. **Thomas Braun** of the **Max Planck Institute for Heart and Lung Research** in Bad Nauheim is studying the amphibians to learn how an organism can regrow entire organs. Perhaps one day it will help enhance the capacity for regeneration in humans.

TEXT **STEFANIE REINBERGER**

Bad Nauheim – a small Hessian town that is a shining example of art nouveau, mineral springs, spas and clinics. The town's website touts "innumerable little natural wonders" and promises health, recovery and complete renewal. However, few people would guess that the biggest regeneration potential can be found in the basement of the Max Planck Institute for Heart and Lung Research at the edge of the spa gardens. Two basement rooms hold countless aquariums, terrariums and aquaterrariums that house more than a thousand specimens of the eastern newt (*Notophthalmus viridescens*).

The amphibians – which, incidentally, aren't green as young animals, as their Latin name implies, but orange-brown with reddish spots – have a remarkable self-healing capacity. They're able to regrow a severed leg or damaged eye lens completely within a few months. Even the heart is able to renew itself completely after injury.

Humans can only dream of such self-healing powers. Only a few organs in our body, the liver and skeletal muscles, are able to regenerate to any appreciable extent; blood cells are also constantly renewed. The heart, though, is an entirely different matter: After a heart attack, dead heart muscle cells aren't renewed to a sufficient degree. Instead, scar tissue forms. The jury is still out on whether the pumping organ in our chest has a reservoir of stem cells that can develop into new heart cells.

Breeding the amphibians from North America is a complicated undertaking. They require different conditions depending on their stage of development. As larvae, they live in water, as young animals on land, and in adulthood they dwell both on land and in water. "In recent years we've observed that the adult animals feel more comfortable when they can walk on land and not just on a floating island," says Miroslaw Grala. The keeper lovingly drapes moss and

Photo: Frank Vinken

The eastern newt is one of the most common newts of North America, where it occurs mainly in moist deciduous and coniferous forests. The greenish tinge from which the newt's Latin name is derived is displayed only by older animals when they reach sexual maturity.

lays fine white gravel in a freshly cleaned aquaterrarium that will soon serve as a newt home.

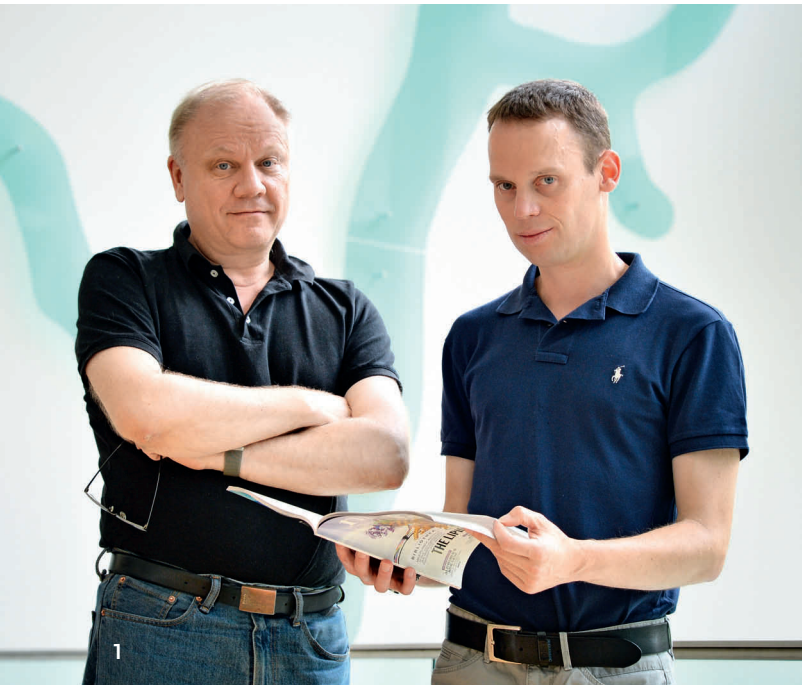
"Since we started providing adult animals with such aquaterraria, they've reproduced better," says Grala. He's clearly proud of his protégés – and not just because of the fact that the researchers based in Bad Nauheim have been the first in the world to succeed in breeding the newts in their own institute. "Although it takes a great deal of work, it's also a lot of fun," says Miroslaw Grala. "I think the animals are simply fascinating."

Master of the newts is Thomas Braun, Max Planck Director and head of the Cardiac Development and Remodeling Department. He began working with the eastern newts in the 1990s at Martin Luther University in Halle-Wittenberg. "It was completely uncharted territory back then. We simply went to a hardware store and bought aquariums, ordered newts from breeders in the US and gave it a try."

The animals are still exotics among laboratory animals. Thomas Braun therefore has mixed feelings about research with the amphibians. "They're truly fascinating model organisms for studying organ regeneration. But from a technical point of view, they're a nightmare." Only around 15 research groups in the entire world are working with these animals. That makes life difficult for the scientists in Bad Nauheim.

Whereas copious information and technical know-how are available for common laboratory animals such as mice, fruit flies, zebra fish and the nematode *Caenorhabditis elegans*, there are no standardized laboratory protocols for working with the eastern newt – not even information on how best to keep and breed them.

What's even worse for Thomas Braun is the fact that the newts' genome hasn't been sequenced and won't be in the foreseeable future. The analysis of a genome that is about ten times larg-

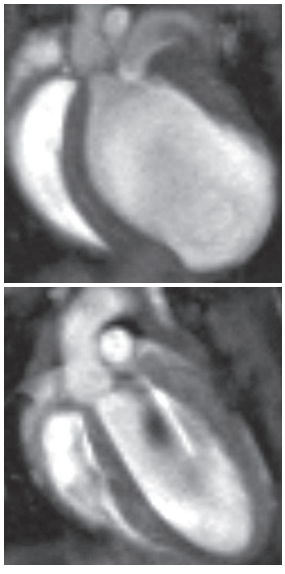


1: Together with their colleagues, Thomas Braun (left) and Jochen Pöling discovered the chemical messenger oncostatin M, which controls the reverse development of muscle cells. 2: Although the preferred tool of the bioinformatician Mario Looso is the computer, during his doctoral work he also studied newts intensively. 3: The amphibians thrive and multiply in the institute's aquaterraria. 4: Susanne Kreutzer is an expert in injecting DNA into the nucleus of egg cells, allowing Jochen Pöling to investigate how the loss of a gene affects the ability to regenerate.

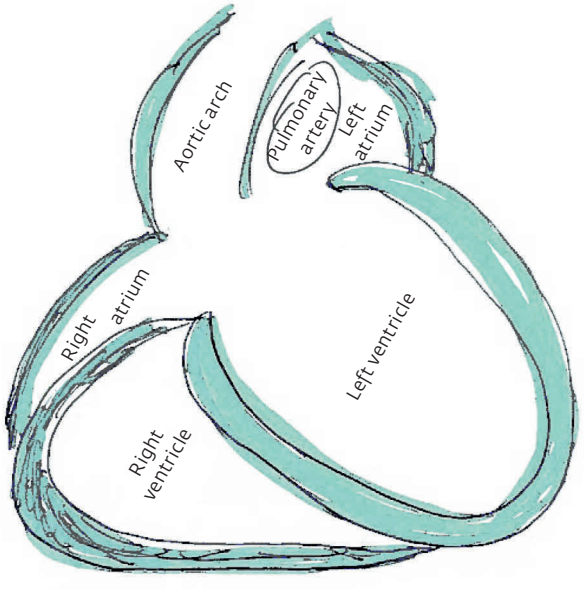
er than that of humans would be an enormous challenge, even with modern methods. "Without this information, however, we're unable to switch off genes and investigate what effect they have," says Braun. "And until we know which genes are involved in the regeneration of tissue, we can only observe and describe the self-healing processes in the newt – but we won't really understand exactly what's going on."

Braun and his colleagues have thus sought other ways to identify newt genes involved in regeneration. Instead of sequencing the entire genome, the researchers initially focused on those sections of DNA that are active and are expressed during development from the larval to the adult stage and during regeneration. To this end, they isolate messenger RNA (mRNA, the molecules that store genetic information before it is translated into proteins) from larvae and damaged tissue from adult animals. They then compare such samples with those of healthy adult animals.

The outcome of such investigations is initially a bewildering mass of data. Bioinformatician Mario Looso's job is to



MRI scan of a mouse heart: After an infarction, the left ventricle (in the top image on the right) is significantly larger than in healthy hearts (bottom). In addition, the myocardium is thinner due to passive dilation. Many heart muscle cells in the infarcted area have died, so that the heart's pumping capacity is drastically reduced. In contrast to the mouse, a person with such a damaged heart couldn't survive.



Photos: Frank Vinken (top left), MPI for Heart and Lung Research (top right and bottom)

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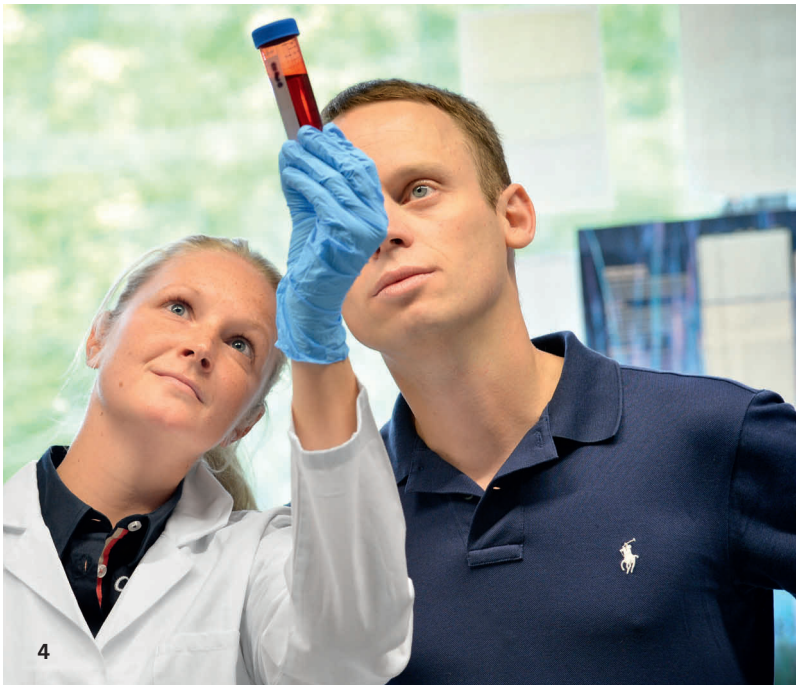

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extract meaningful data from this flood of information. Instead of a pipette, his tool of the trade is a computer. Although Looso's interests were originally mathematics and the development of databases, he has long been intrigued by newts. He isn't in the least put off by the challenge – quite the contrary. "That's precisely the attraction for me as a bioinformatician," he says. "I have to devise suitable methods and am able to develop a newt database from scratch."

PROTEINS FOR SELF-HEALING

In this way, the scientists in Bad Nauheim have identified nearly 15,000 messenger RNA molecules in the eastern newt that are translated into proteins. Of those proteins, 830 were previously unknown and apparently occur only in the newt – an astonishingly large number for a single species. Moreover, some of the new proteins belong to a previously unknown protein family that may play a key role in the self-healing capacity of the amphibians. In fact, the researchers have discovered protein families that control regeneration of the eye lens and heart.

A particularly exciting candidate is a protein called nsCCN, a member of the CCN family of proteins. These proteins stimulate cell growth and occur in very similar form throughout the animal kingdom – from zebra fish to humans. One representative, CCN4, for example, is active in mammals when the heart repairs itself to some extent after damage.

It's possible that nsCCN is involved in the process when newt cells undergo reverse development. This is the very trick that newts employ. "The amphibians don't regenerate defective tissue or amputated limbs from stem cells to any meaningful extent," explains Braun. "Instead, cells that are already specialized regress, so that they behave much like stem cells." Researchers call this process dedifferentiation. Heart muscle cells, for example, lose their specialization and begin to proliferate at an increased rate until a sufficient number of new cells have formed that will mature into new heart muscle cells.

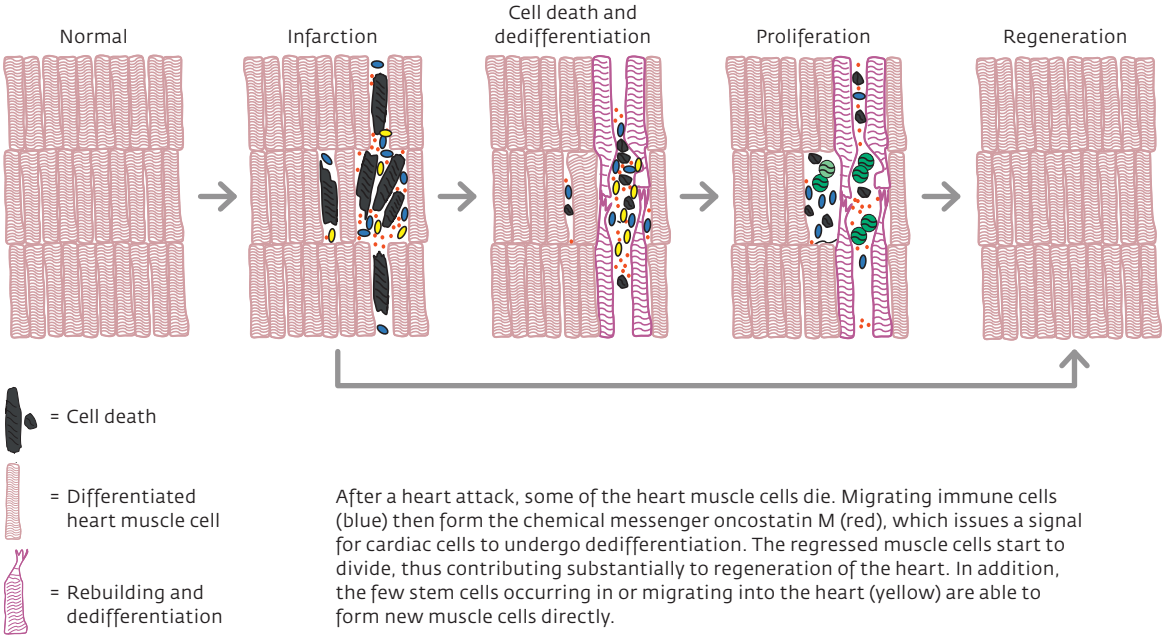
Thomas Braun suspects that heart muscle cells in humans also simply shift into reverse gear. His team has discovered such dedifferentiated cells in the myocardium following a heart attack or chronic oxygen deficiency. This also explains why researchers find so few cardiac stem cells despite the fact that damaged cells are still partially replaced after a heart attack.

Another remarkable observation regarding damaged cardiac muscle is that, after a heart attack, inflammatory cells migrate into the heart. This also happens in newts and is essential for the amphibians to recover quickly and regenerate injured body parts. Is this the key to self-healing? Do the migrated cells release a substance that promotes dedifferentiation?

ONCOSTATIN M PROMOTES REGENERATION

Jochen Pöling, who also works as a surgeon at the Heart Center of Schüchtermann Hospital in Bad Rothenfelde in Lower Saxony, is therefore searching for such chemical messengers released by inflammatory cells. Together with his colleagues, he observed that, shortly after an infarction, the chemical messenger oncostatin M occurs in damaged tissue, where it stimulates cellular dedifferentiation. In genetically modified mice lacking fully functional oncostatin M, fewer heart muscle cells revert to their original undifferentiated state after an infarction. Moreover, they

Photos: Frank Vinken (2)



After a heart attack, some of the heart muscle cells die. Migrating immune cells (blue) then form the chemical messenger oncostatin M (red), which issues a signal for cardiac cells to undergo dedifferentiation. The regressed muscle cells start to divide, thus contributing substantially to regeneration of the heart. In addition, the few stem cells occurring in or migrating into the heart (yellow) are able to form new muscle cells directly.

are more likely to die from the consequences of the infarction.

CHEMICAL MESSENGERS ADHERE TO OTHER RECEPTORS

High oncostatin M levels could therefore stimulate the reverse development of cardiac cells and activate repair mechanisms in the heart. Unfortunately, things aren't that simple. In humans, the signaling molecule also binds to other receptors, leading to, among other things, an increased tendency for the blood to clot – clearly a counterproductive effect for infarction patients.

The effect of the chemical messenger is also ambivalent in mice. Although oncostatin M and the dedifferentiated heart muscle cells initially protect the heart after an acute myocardial infarction, this is partly due to the fact that the dedifferentiated cells are much more able to cope with oxygen deficiency. "However, the longer oncostatin is released and the cells dedifferentiate, the more the function of the myocardium deteriorates," Pöling explains.

The Max Planck researchers have identified a potentially key difference between the newt heart and the mammalian heart: whereas, in newts, it is primarily new heart muscle cells that form from dedifferentiated cells, in mice and humans it is mainly scar tissue. These cells aren't muscle cells and therefore don't increase the pumping performance of the heart after a heart attack. But why does dedifferentiation result in apparently useless scar tissue in humans, yet generate new healthy cells and even entire organs and limbs in newts?

It's possible that the scar tissue isn't as superfluous as it might appear at first glance; it could, in fact, serve to stabilize the heart. "The newt ramps down its entire metabolism and blood pressure during regeneration. In this way, its heart is able to undergo remodeling without bursting," Braun explains. The human heart, in contrast, has to do a lot more pumping work. If repair work on the heart is too extensive, the heart would become unstable and would be unable to withstand the pressure.

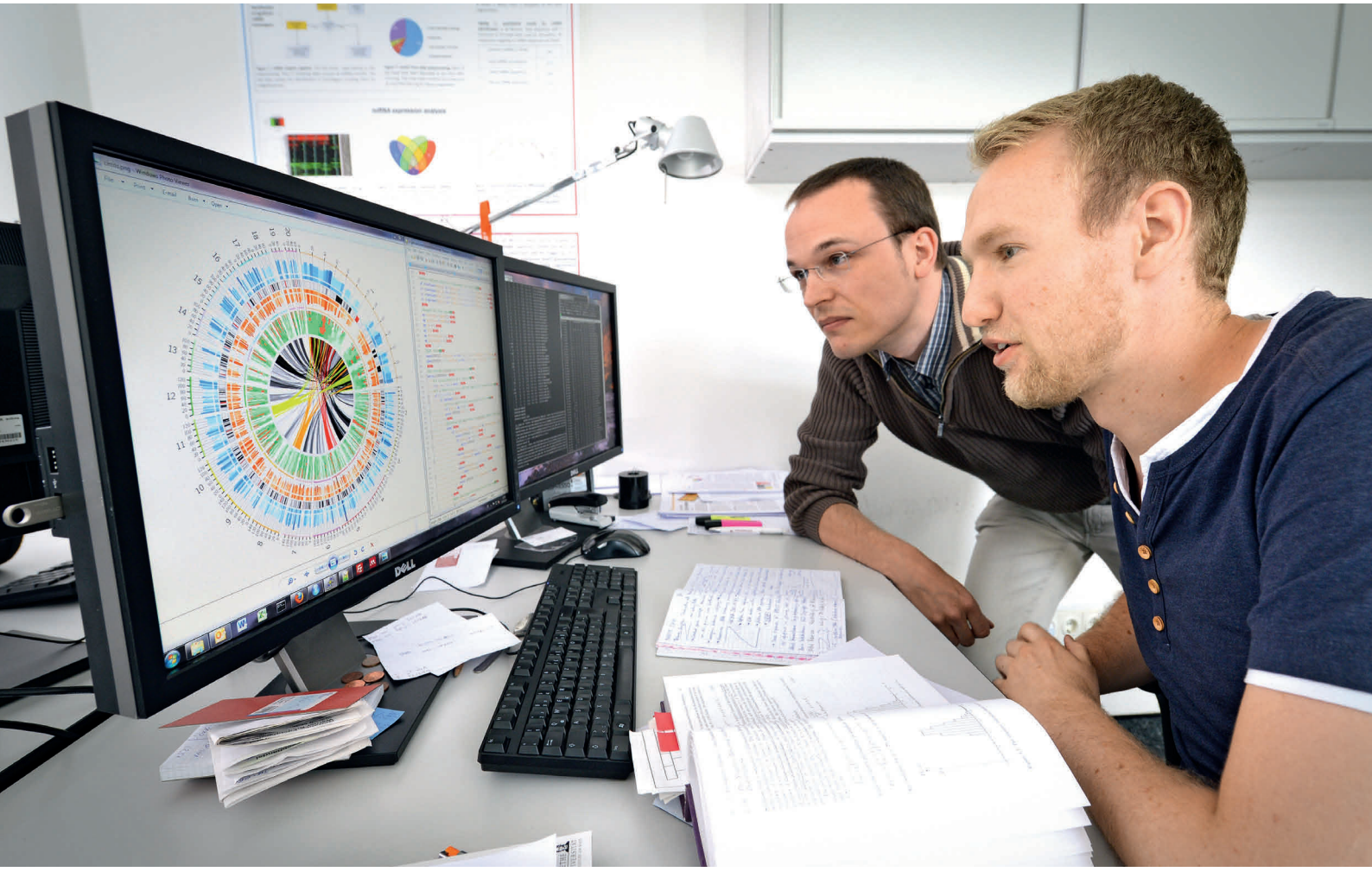
The observations in newts thus can't be readily applied to mammals. Nevertheless, the discovery of oncostatin M has taught the scientists something important: although scar tissue, which is so feared by doctors, can't replace myocardial tissue and improve the heart's pumping capacity, it does protect the heart from cellular stress due to oxygen deficiency and lends it a certain stability. Scarring is, in effect, a form of damage limitation.

TIMING IS CRUCIAL

The processes that occur in the heart after an infarction thus perform a balancing act between protection and loss of function. The researchers therefore describe oncostatin M as a Janus-faced protein. In this sense, despite all the adverse effects, the signaling substance could point the way to new forms of treatment – just a different one than the researchers had originally expected.

Furthermore, timing is crucial. In the early phase after a heart attack, it might be helpful to stimulate the secretion of

Graphic: MPI for Heart and Lung Research



Analysis of gene activity: A computer program compares messenger RNA molecules and their functions in the newt with those of other experimental animals. It also shows bioinformaticians Carsten Künne (left) and Jens Preußner the amount of RNA and its occurrence in various tissues.

oncostatin M and thus dedifferentiation. This would help protect heart muscle cells and facilitate cellular repair. Thomas Braun also hopes that additional – as yet unknown – chemical messengers can stimulate the dedifferentiated cardiac cells to divide. However, in a later post-infarction phase, the mediator would have to be blocked to prevent progressive dedifferentiation and scarring.

"Perhaps after an infarction it's all a question of finding the right balance between acute emergency repair and the gradual progressive loss of the heart's pumping function," explains Braun. A balance that the tiny regeneration artists in the basement of the Max Planck Institute in Bad Nauheim discovered a long time ago. ◂

Photo: Frank Vinken

TO THE POINT

- **The eastern newt regenerates damaged tissue and amputated limbs only to a limited extent from stem cells. Instead, already specialized cells regress and begin to divide until enough new cells have been formed to regrow specialized tissue.**
- **In the newt, these dedifferentiated cells in the heart give rise primarily to new heart muscle cells. In mice and humans, it is mainly scar tissue that forms. Although this tissue can't support the heart's pumping action after a myocardial infarction, it protects the heart against oxygen deficiency and stabilizes it. In many patients, however, this later results in heart failure.**

GLOSSARY

Cardiac stem cells: Cardiac stem cells divide continuously during development from embryo to adult animal until a cluster of cells gives rise to a complex organ comprising ventricles, atria, heart valves and coronary vessels. Once this process is complete, most stem cells are switched off. But even in the adult mammalian heart, some stem cells remain. These cells divide throughout life to form new heart muscle cells. However, they are very few in number: within one and a half years, only about 5 percent of heart muscle cells are likely to be renewed – not enough to heal a damaged myocardium, for example after a heart attack.

Regeneration: Many animals are able to regenerate damaged tissues and organs. However, while simpler organisms such as jellyfish and flatworms are able to form a whole new organism from parts of the body, few vertebrates are able to regrow multiple organs. The masters of regeneration among vertebrates are newts and salamanders, followed by other amphibians, fish and – lagging behind by some distance – mammals.