Nature **as a Pharmacy**

Bacteria, plants and animals are full of unknown substances that could be beneficial for humans. At the **Max Planck Institute of Molecular Physiology** in Dortmund, **Herbert Waldmann** tests natural products for their biological efficacy and tries to mimic their effects with simpler molecules.

TEXT **CATARINA PIETSCHMANN**

Evolution has endowed many species with substances that enable them to communicate, attract partners or deter enemies. Some of those substances have also proved to have powerful effects in humans. Taxol, a compound discovered in the bark of the Pacific yew, is one example. And Germanic tribes were already aware of the analgesic effect of salicylic acid obtained from willow bark.

Atropine, a substance from deadly nightshade that blocks nerve cell receptors; morphine from the opium poppy; and penicillin antibiotics from various fungi – the list of small molecules with potent effects is endless. "Almost a third of our drugs are still derived from natural products, and another third are proteins," says Herbert Waldmann, Director of the Department of Chemical Biology at the Max Planck Institute of Molecular Physiology.

In the 1990s, a scientific field known as combinatorial chemistry was used to synthesize a large number of chemical compounds. Hundreds of thousands of compounds were collected in substance libraries and screened in high-throughput tests for their pharmacological effects on cells.

TOO FEW NEEDLES IN THE HAYSTACK

The sobering conclusion was that just one in one hundred thousand compounds has a biological effect, corresponding to a hit rate of just 0.001 percent. Now, you might argue that, given one million candidate compounds, you would at least have ten hits. "Unfortunately, this calculation was not borne out," says Waldmann. "Continuously expanding the substance libraries isn't the solution." An ever-growing haystack doesn't automatically contain more needles.

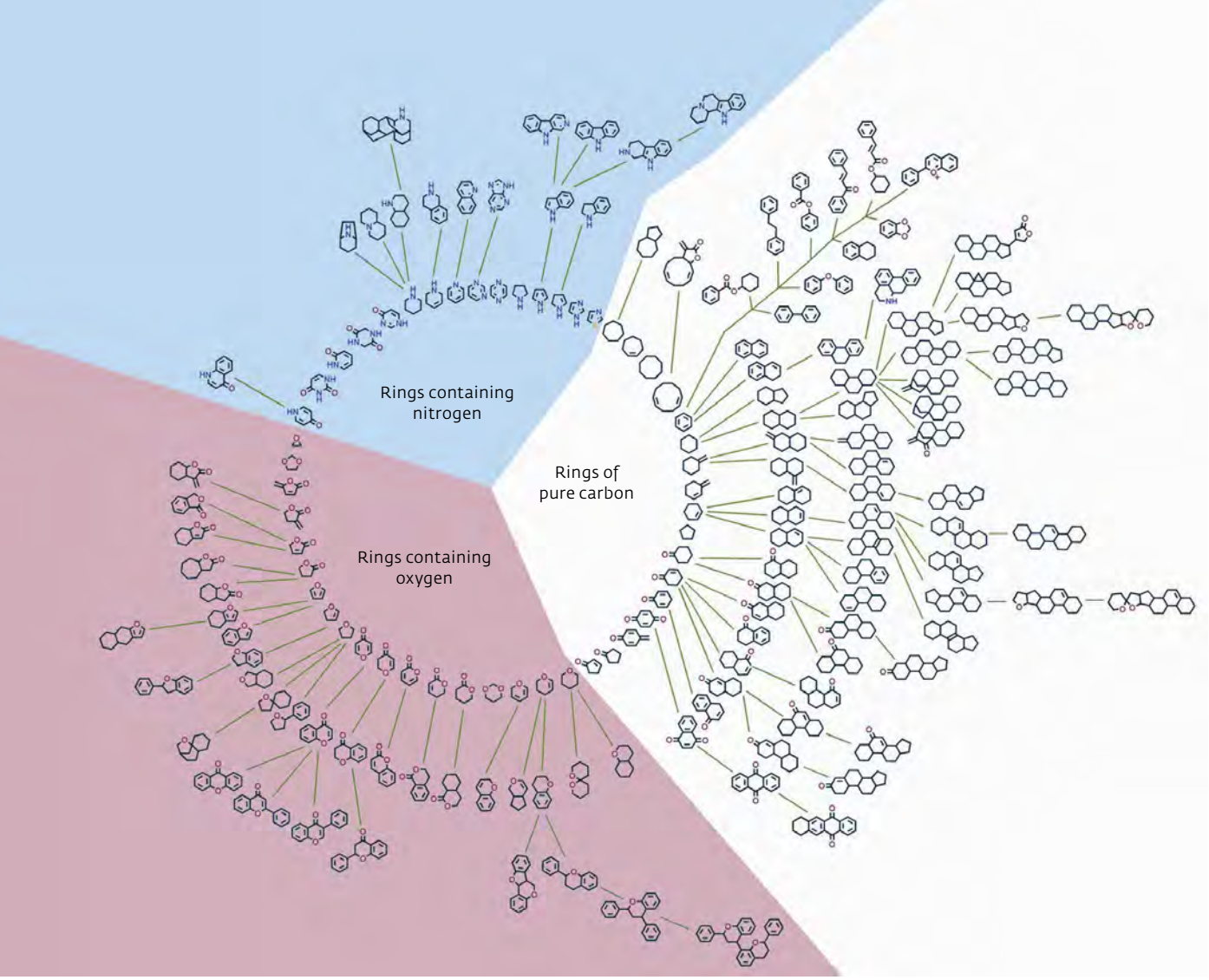
The synthesis of complex natural compounds is still an exciting challenge for chemists. It also marked the start of Waldmann's scientific career. "But this form of drug discovery is slow, and multi-stage chemical syntheses are often unsuitable for producing substances on a large scale for industry."

Waldmann therefore took a different approach. Instead of randomly synthesizing and screening chemical compounds, he let himself be guided by chemical structures that have already proved effective. "We are trying to understand what is essential in the structures of natural products and to use those findings to synthesize new compounds," Waldmann explains.

A drug works because its active substance binds to the active center of a protein, altering or completely block-

The pain-relieving and fever-reducing effects of an extract of willow bark were already known in antiquity. The effects are due to the presence of salicin, which the body converts into the active substance salicylic acid. It is the lead structure of the even more potent and safer compound acetylsalicylic acid (ASA), the active substance in aspirin.

Photo: Shutterstock



Molecular tree: The chemical structure of the natural substances at the twigs can be simplified in a step-by-step process. In this way, researchers can trace along a branch to a basic structure that still exhibits similar biological activity to the original compound but that can be more easily synthesized in the lab. The computer program Scaffold Hunter, which was developed in Waldmann's department, is based on this principle.

ing the protein's activity in the process. It is striking that nature uses only a modest number of proteins from all that are theoretically possible. In purely mathematical terms, 10^{390} proteins with a length of 300 amino acids could be synthesized from the 20 naturally occurring amino acids. Yet even the genomes of highly developed organisms contain the blueprints for no more than a hundred thousand proteins.

Nature is just as parsimonious when it comes to protein folding. "There are probably no more than several thousand folding types," Waldmann speculates. "This makes sense – after all, nature doesn't need to constantly reinvent the wheel." As a result, a substance from bacteria is effective in humans because it fits into the binding pocket of a protein that the common ancestor of bacteria and humans already largely possessed hundreds of millions of years ago.

The active molecules that occur in nature are likewise limited. They usually consist of up to nine interlinked ring systems, but most natural substances have only two to four rings. With this format, they evidently fit into the binding pockets of most proteins. Although the basic shape and size of the binding pockets thus vary within only a narrow range, their chemical "lining" is highly variable. "How selectively a drug binds depends on the side chains of the proteins and the functional groups of the natural substances."

Waldmann's strategy for discovering new drugs is to simplify the chemical scaffolding of the natural products to such an extent that a compound is just barely effective. He then approaches the effectiveness of the original by attaching functional groups to the molecule. In this way, he doesn't have to deal with large, complex molecules and can instead concentrate on smaller ones that are easier to synthesize. "What's the use of even the most effective natural substance if its complicated structure means that only a few crumbs can be produced? It might be the savior of humankind, but if 100 kilograms of it were needed, it would still be useless," Waldmann says.

First, he needed an overview of the diversity of the structures of natural substances – a Herculean task that would be impossible without the help of a computer. Waldmann's team wrote a software program and analyzed all 190,000 natural products

Graphic: Max Planck Institute of Molecular Physiology

Photo: Frank Vinken

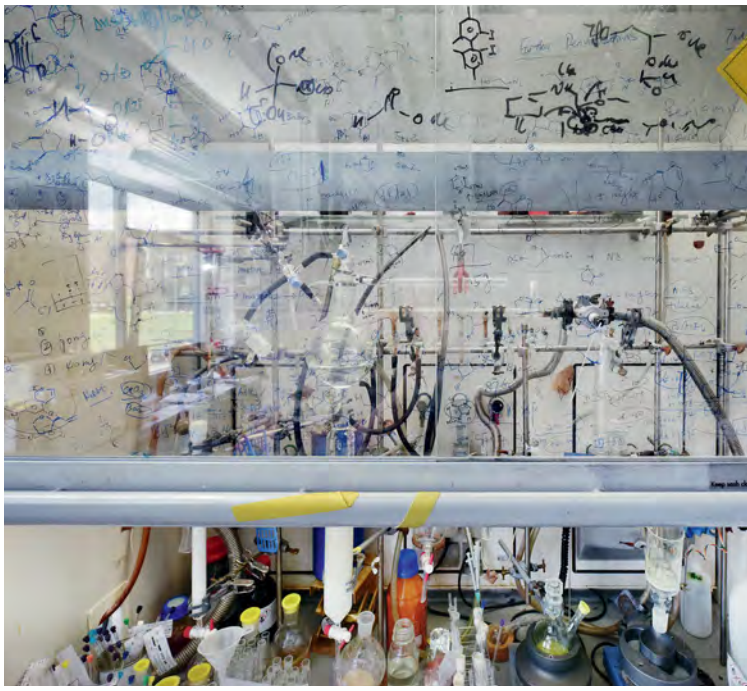
Tracking down new drugs: Taking nature as a model, Herbert Waldmann is utilizing naturally occurring molecules to develop new substances for medicine that are easier to synthesize and more effective than their natural counterparts.

known at the time. They then trimmed them back to their chemical backbone and sorted them according to complexity and structural kinship. The result was a tree-like structure. The most complex, but often most effective, nodes are located on the outermost branches. Toward the trunk, the structures become simpler and their biological activity weaker.

The trunk is formed by the simple molecular rings arranged by size and divided into sectors of pure carbon rings and oxygen- and nitrogen-containing rings.

FROM COMPLEX MOLECULES TO SIMPLE ONES

Scaffold Hunter, as Waldmann named the program, is available to scientists and pharmaceutical companies the world over free of charge. The software contains – if known – the biological activity of the natural products stored in it. If a researcher has found an active substance corresponding to a particular disease, he or she can trace the branch from the complex original structure toward the trunk to find a simple compound that can easily be synthesized and optimized. Because such a basic compound is simpler in structure than a natural product, it tends to be less selective and can bind to multiple proteins. The drug candidate then has to be tweaked so that it binds exclusively to the target protein. Otherwise, it could trigger severe side effects and would be useless as a drug. >



Left For screening purposes, a robotic dispenser applies solutions to test plates. Instead of a test liquid, an orange dye is applied from the device's supply tank to check the accuracy of the instrument, which can then be calibrated.

Right Glass extraction shafts in the labs prevent gaseous substances from escaping from the apparatus. The researchers sometimes scribble chemical formulas on the front panels of the extractors.

Waldmann's team has in fact had significantly greater success with this approach. Instead of 0.001 percent, their hit rate is now around 1 percent. "When we make 200 variants of a selected class of substances, an average of two turn out to be useful. After the second optimization round, we usually have 20 to 30 potent molecules," Waldmann says.

Things become especially interesting where there are gaps in the structural tree of natural products. For example, if a substance with four rings is biologically active and one with two rings is as well, it can be surmised that a compound with three rings will also be active. "We tested that premise and confirmed it in cell tests. The program is therefore able to predict the biological activity of substances," Waldmann explains.

Herbert Waldmann's research would hardly be possible without extensive cell tests, known as screenings. In creating the Compound Managing and Screening Center (COMAS) at the Max Planck Institute in Dortmund, the Max Planck Society merged its previously scattered substance libraries. The combined library now contains over 250,000 chemical compounds. Most of them were acquired from other suppliers, but 10 percent of them come from Max Planck laboratories. They don't exist anywhere else in the world. Every Max Planck scientist can use the Center and test substances for their effects.

SUBSTANCES FOR CANCER RESEARCH

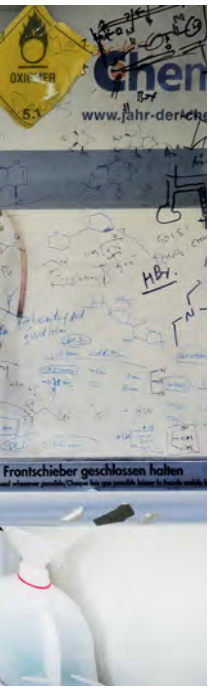
Some of the samples are from Waldmann's own department. His team has used Scaffold Hunter to synthesize its own library of substances based on natural products. The scientists use the program mainly to search for cancer drugs, taking a biological hypothesis as their starting point.

An example: "Cancer cells need large amounts of nutrients to grow. If we block the channel proteins in the cell membrane through which, for instance, sugar enters, we could starve the cancer cells. So we searched for an inhibitor of those membrane channels in our library – and our efforts paid off." The researchers then optimized the inhibitor and handed it over to the Lead Discovery Center next door.

The Lead Discovery Center was founded in 2008 on the initiative of the Max Planck Society to bridge the gap between basic research and industry. The Center's scientists test the efficacy, uptake and tolerance of promising drug candidates in animals and, if necessary, improve those properties. If the tests are satisfactory, a pharmaceutical company can purchase the license for the active substance and carry out the necessary clinical tests.

Very few candidates successfully make it past the Lead Discovery Center. Englerin, for example, a plant-derived natural substance that selectively kills renal cancer cells in cell tests, failed the

Photos: Frank Vinken (2)



Left The cells grow in the liquid-filled wells of the plates at a temperature similar to that of the human body. This allows researchers to directly test the effects of substances from the Center's library of compounds.

Right Sonja Sievers heads the Screening Unit of COMAS. Using a modern robotic system, she is able to screen a large number of chemical compounds for biochemical and cellular effects.

first experiments in animals. "The mice died within five minutes, because englerin blocks a calcium channel not only in cancer cells but also in lung tissue, resulting in massive edema," Waldmann says.

Substances that block the Ras signaling pathway in cancer cells have long been right at the top of Waldmann's agenda. They attracted his attention as early as the 1990s while he was still working in Bonn. A mutation leads to Ras proteins, which are anchored in cell membranes via their fatty acid residues, giving cancer cells a signal to undergo division. Mutated Ras proteins play a role in one third of all cancers.

Waldmann began to develop active anti-Ras substances and, in doing so, reached "the natural limit of chemists," as he puts it. "You've synthesized a molecule – and then what? You open the fridge door, put the substance in, close the door … and move on to the next project?" That wasn't enough for Waldmann. He searched around for someone who knew more and discovered Alfred Wittinghofer, who was then head of the Structural Biology Department at the Max Planck Institute in Dortmund. Wittinghofer was studying the mode of action of these signaling proteins together with cell biologist Philippe Bastiaens, now also Director at the Institute.

TRACKING RAS TOGETHER

"Wittinghofer was, so to speak, 'Mr. Ras'. And I thought to myself: you should call him," Waldmann recalls. This was the start of a highly successful collaboration between the two scientists. In 1999, Waldmann himself was appointed to the Max Planck Institute, where he now heads the Department of Chemical Biology, and Bastiaens came to Dortmund a few years later. From that point on, the three departments complemented each other. Wittinghofer and Bastiaens unraveled how Ras is transported in cells. What the researchers then needed was a molecule that blocks this transport process. That's where COMAS came into play. "Together with COMAS, we developed a cell test and combed through our library. We then further optimized the prime candidates in the lab."

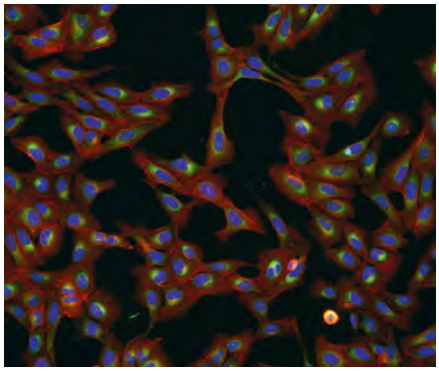
One of those candidates was deltarasin, which blocks Ras transport and therefore the signal pathway. Unfortunately, however, it also disrupts other processes, causing serious side effects. Other candidates, on the other hand, produced fewer side effects but weren't effective enough. Cancer cells even succeed in reversing the Ras blockade. Meanwhile, the Max Planck scientists are developing the fourth generation of blockers that dock so tightly to their target protein that cancer cells are unable to remove them.

Waldmann has high hopes for a technique known as cell painting. He plans to develop a cell test that will allow him to say right after the synthe-

Photos: Frank Vinken (2)

Below Cell painting is a technique for staining cellular organelles such as the nucleus (blue), the endoplasmic reticulum (green) and the cytoskeleton (red). If a substance acts on one of these organelles, it will produce an altered color pattern.

Right System operator Miriam Kunkel curates the COMAS substance library, which contains more than 250,000 compounds. Temperatures of around minus 20 degrees Celsius ensure that the molecules remain intact even after prolonged periods.



sis: "We don't know what the molecule can do yet – but we do know that it has potential!"

Next, Waldmann would like to go beyond what nature can do. "We know that the number of basic chemical structures in nature is limited. So far, we have only mimicked what nature has already achieved, so we only find the bioactivities of those natural products, never anything completely new." Waldmann therefore wants to break down the molecular structures found by Scaffold Hunter into smaller fragments. "Then we can reassemble them in ways that nature has never done!" This will create substances that look like natural compounds but aren't: pseudo-natural substances – "nature 2.0", as it were.

But will it work? Waldmann nods. They have already been successful on two occasions – not enough to be able to make a definitive statement, but the signs are good that his drug libraries will soon be enriched with new, promising substances. ◂

TO THE POINT

- **Using a software program, scientists are able to strip the chemical structure of a biologically active natural product down to its basic molecular scaffolding. Such products are easier to synthesize and, if necessary, optimize in the lab.**
- **By combining individual structural elements of natural products, scientists hope to create chemical compounds with completely new properties.**
- **A number of institutes have grown up around the Max Planck Institute of Molecular Physiology in Dortmund to explore potential new drugs and pave the way for their use in medicine.**

GLOSSARY

Cell Painting: A technique that involves labeling various signaling pathways or cell organelles with fluorescent dyes, resulting in a multicolored pattern. For drug screening, the colorful cells are distributed across multiple test samples and exposed to substances whose effects on cells are well known. These reference substances change the color patterns in a characteristic way. The effects of a new, unknown molecule can then be derived by comparing the color patterns.

COMAS: The aim of the Compound Management and Screening Center is to exploit scientific findings from basic research by the Max Planck Society for medical research and the development of new therapeutic applications. Each substance in the substance library, which currently holds 250,000 compounds, is stored in barcoded tubes at minus 20 degrees Celsius. Tiny amounts of substance – just a few billionths of a liter (nanoliters) – are sufficient for the screening tests. Substances that show promise in the tests at COMAS are passed on to the Lead Discovery Center, where they are refined for medical applications.

Photos: MPI of Molecular Physiology (left); Frank Vinken (right)

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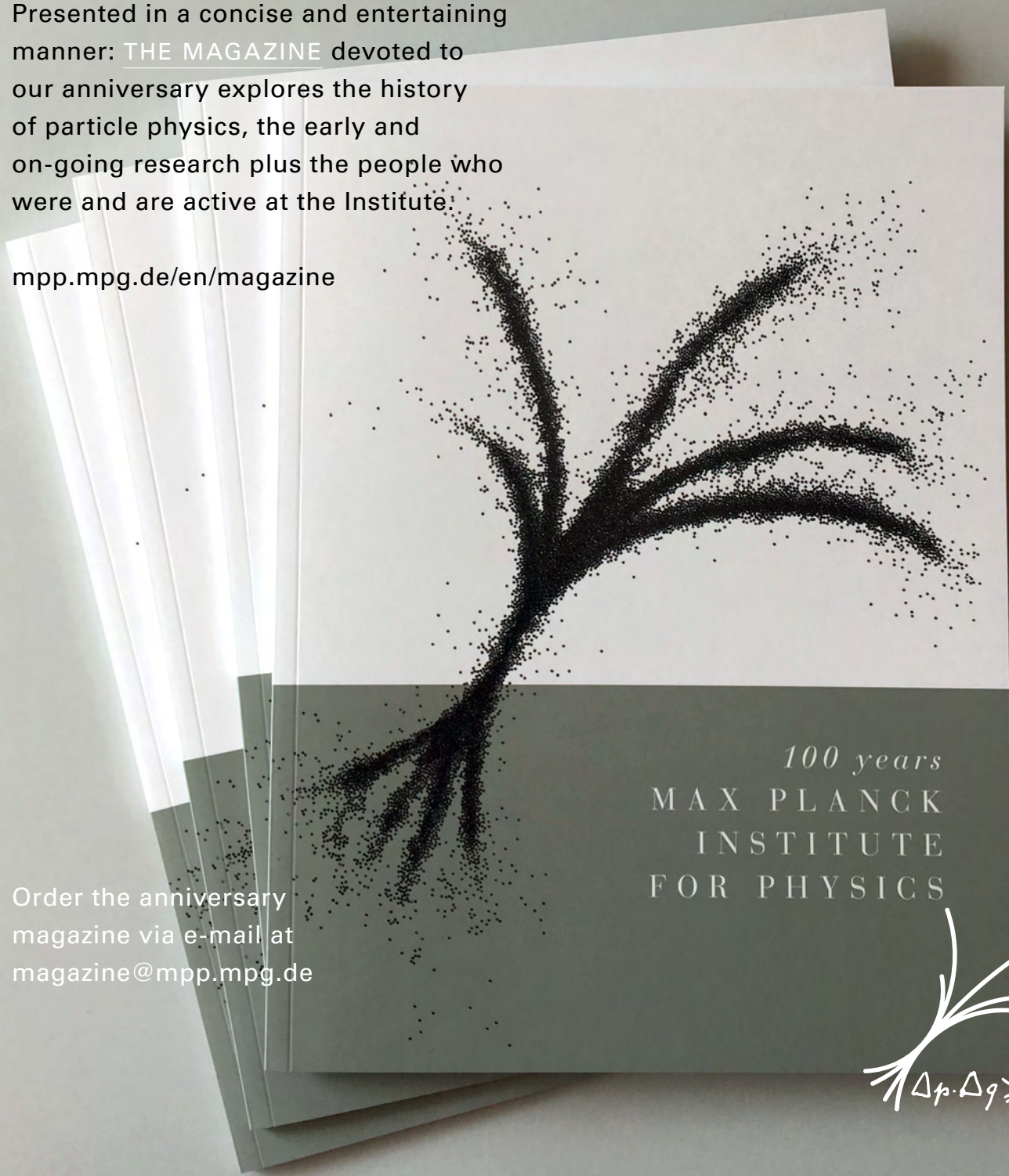

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