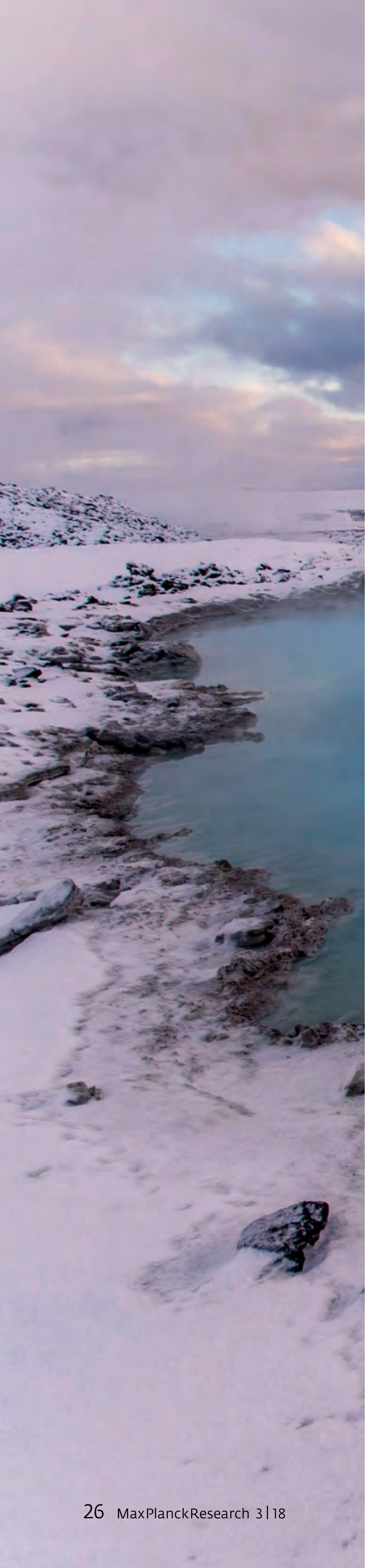


Elixirs from the **primordial soup**

In the Bible, the universe was created step by step: first light, then water and land, and finally the terrestrial animals and humankind. However, from a scientific viewpoint, it seems that the building blocks of life might not have come into being successively, but rather at the same time – at least, this is what **Hannes Mutschler** of the **Max Planck Institute of Biochemistry** believes. He and his colleagues in Martinsried, near Munich, are researching the role played by RNA molecules in the emergence of life.

TEXT **CLAUDIA DOYLE**

Let us go back on a journey through time to the beginnings of our planet. Around 4.5 billion years ago, the Earth was an extremely inhospitable place: its surface was an incandescent sea of molten stone, the sky was full of meteorites that rained down incessantly from space and ripped deep craters in the Earth, and the atmosphere was a mixture of carbon dioxide, ammonium and methane. Living organisms? Not a trace – not in this inferno.

Over the next billion years, the fiery turmoil subsided. The Earth cooled down; oceans and continents formed. And somewhere in the water, life began: the elements of carbon, hydrogen and nitrogen bonded to form complex molecules, and the first cells came into being. Yet how exactly did all this come about?

“It goes without saying that we can’t turn back time. That’s what makes it so hard to find out what really happened,” says Hannes Mutschler, who leads the Biomimetic Systems research group at the Max Planck Institute of Biochemistry. “This is why we are trying to recreate the origins of life in the lab – in conditions like those that prevailed on Earth at the time.”

CELLS CREATED BY HUMAN HAND

He and his colleagues aim to create artificial systems that behave like living cells. There are two different ways to go

Photo: Christian Frumolt/Look Foto

Fluctuations between heat and cold may have contributed to the emergence of life on Earth. Laboratory experiments have shown that repeated freezing and thawing can produce complex RNA molecules that can be passed on in cell-like vesicles. This shows that the first cells may have come into being in hot springs such as those that feed the Blue Lagoon in chilly Iceland.

The conditions on Earth in prehistoric times and those in Hannes Mutschler's working group's laboratory could not be more different, yet the scientists can still research the early stages of life thanks to modern research methods. Left to right: Viktoria Mayr, Kai Libicher, Hannes Mutschler and Laura Weise, Alexander Wagner and Kristian Le Vay.

about this. The first is to take an existing cell and successively remove all the parts that are not essential to life. This method was chosen by American scientist and entrepreneur Craig Venter, who at the beginning of the millennium contributed significantly to the decoding of the human genome. The second method, adopted by Hannes Mutschler, is to reverse the process and create a cell from the bottom up.

AS SIMPLE AS POSSIBLE

Mutschler and his team aim to reconstitute parts of simple "protocells" using the bare minimum required – enzymes, nucleic acid components, and a cell envelope. First, the scientists have to identify the molecular building blocks needed for a cell to be viable. Then they have to assemble them correctly in the test tube. "It's like working with a gigantic Lego set," says Mutschler.

The problem is that nobody is able to say exactly what a minimalistic cell needs in order to live. Over millions of years, evolution has gradually refined numerous processes and interwoven them.

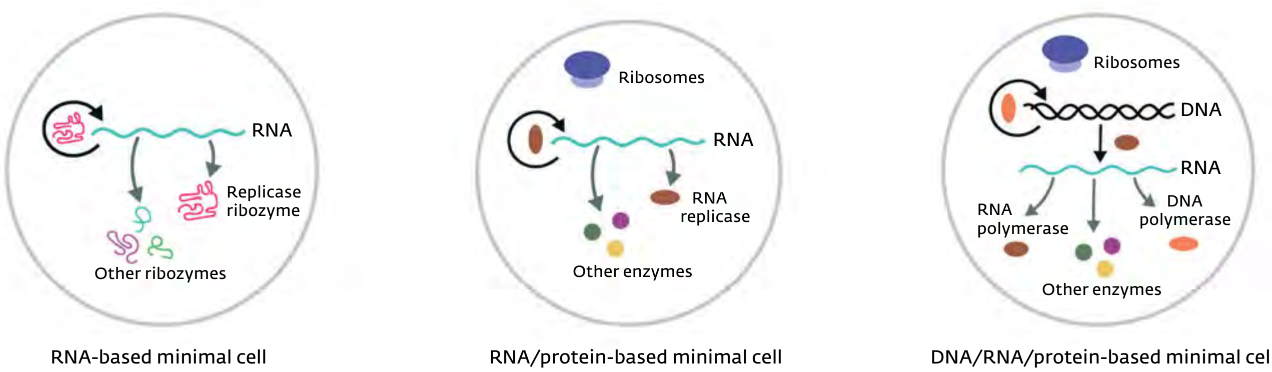
Nowadays, if we look inside a modern cell, it is difficult to distinguish which of the many processes are essential for survival and which are replaceable. "It's as if you would take apart a modern car and be left standing in front of a pile of sheet metal, cables and electronics," says Mutschler. Not all of these components are essential to the functioning of the car, but it is not so easy to see which of them are irreplaceable.

Even in the case of individual molecular structures, it is often unclear which elements are vital. One example of this is the ribosome. This molecular machine uses the cell's genetic information as a template and assembles amino acids in the correct order to form proteins. The structure of the ribosome is highly complex: more than 100 genes are involved in its formation. Is there an easier way? Quite possibly. But nobody yet knows what it is.

Even today, scientists disagree about the origins of life. Some researchers believe that a simple metabolic process was the beginning of everything. After all, living organisms are characterized by their ability to convert energy and use it for their own survival and reproduction. Energy may have been supplied by differences in the concentration of positively charged hydrogen atoms, for example. These so-called proton gradients were created wherever the acidic water in the early oceans came into contact with alkaline water from subterranean hot springs. Genetic analyses have shown that primal metabolic reactions do in fact draw energy from proton gradients.

But can metabolic molecules exist and function in free, unprotected form,

Photo: Axel Griesch



or do they have to be protected and held together by envelopes? After all, what good is it if the first complex molecules form but drift apart in the open sea? For this reason, some researchers believe that tiny droplets consisting of fat molecules acted as the precursors of modern cell membranes, and it was they that made life on Earth possible.

MOLECULAR CLUSTERS INSTEAD OF CELL MEMBRANE

These membranes have to be stable, able to divide, and capable of controlling which substances are transported in and out of the cell. However, cell membranes of the type found in modern-day cells may not have been necessary at first. Small molecular clusters held together by electrostatic forces – known as coacervates – may initially have performed the tasks of cell membranes. One of the goals of Mutschler's research is to prove this hypothesis. In cooperation with two other working groups from the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, the researchers in Martinsried are investigating whether simple biopolymers can exist in coacervates without membrane and whether they are able to catalyze reactions.

In contrast, other researchers believe that life began with the transfer of information from one generation to the next. But how did this transfer take place?

Nowadays, this task is performed by the molecule deoxyribonucleic acid (DNA). DNA doubles whenever a cell divides. Each daughter cell receives a copy of the DNA and thus a full set of genetic information.

However, many scientists think it is unlikely that genetic material originally consisted of DNA. Instead, some suggest that genetic information was conveyed by ribonucleic acid (RNA) molecules, which cells now use as a mediator for the formation of proteins. In a sense, these RNA molecules are DNA copies that migrate from the cell nucleus to the ribosomes in the cell plasma, where they act as templates for the formation of proteins.

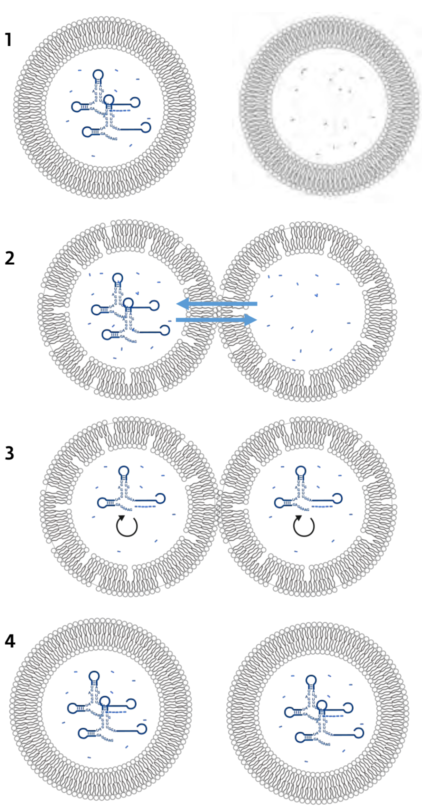
Originally, however, RNA could have been more than an intermediate

Top Different cell variants with minimal components, like those that may have existed when life emerged on Earth: along with RNA-based protocells (left), which use ribozymes to copy their genetic material, there may also have been cells that used proteins (RNA replicase) and ribosomes to replicate their genome (center). Modern DNA/RNA/protein-based cells (right) use DNA as the information carrier for the production of proteins with RNA as the mediator.

Below Part of a chromatography structure with which the researchers can separate mixtures of substances into their components. The individual substances can be divided into different test tubes with the help of the collector.

Photo: Axel Griesch; graphics: MPI of Biochemistry

» In the early days of life on Earth, RNA played a central role by storing information and acting as a biocatalyst. However, RNA molecules probably never acted alone, but were instead supported by other biomolecules.



1 A protocell with (left) catalytic RNA molecules (ribozymes) and without ribozymes (right). **2** The cells exchange their content while in a frozen state. **3 + 4** The ribozymes replicate themselves in the thawed cells. In prehistoric times, simple cell cycles may also have been driven by freezing and thawing, allowing RNA molecules with the ability to self-replicate to spread through the first cells.

Graphics: MPI of Biochemistry

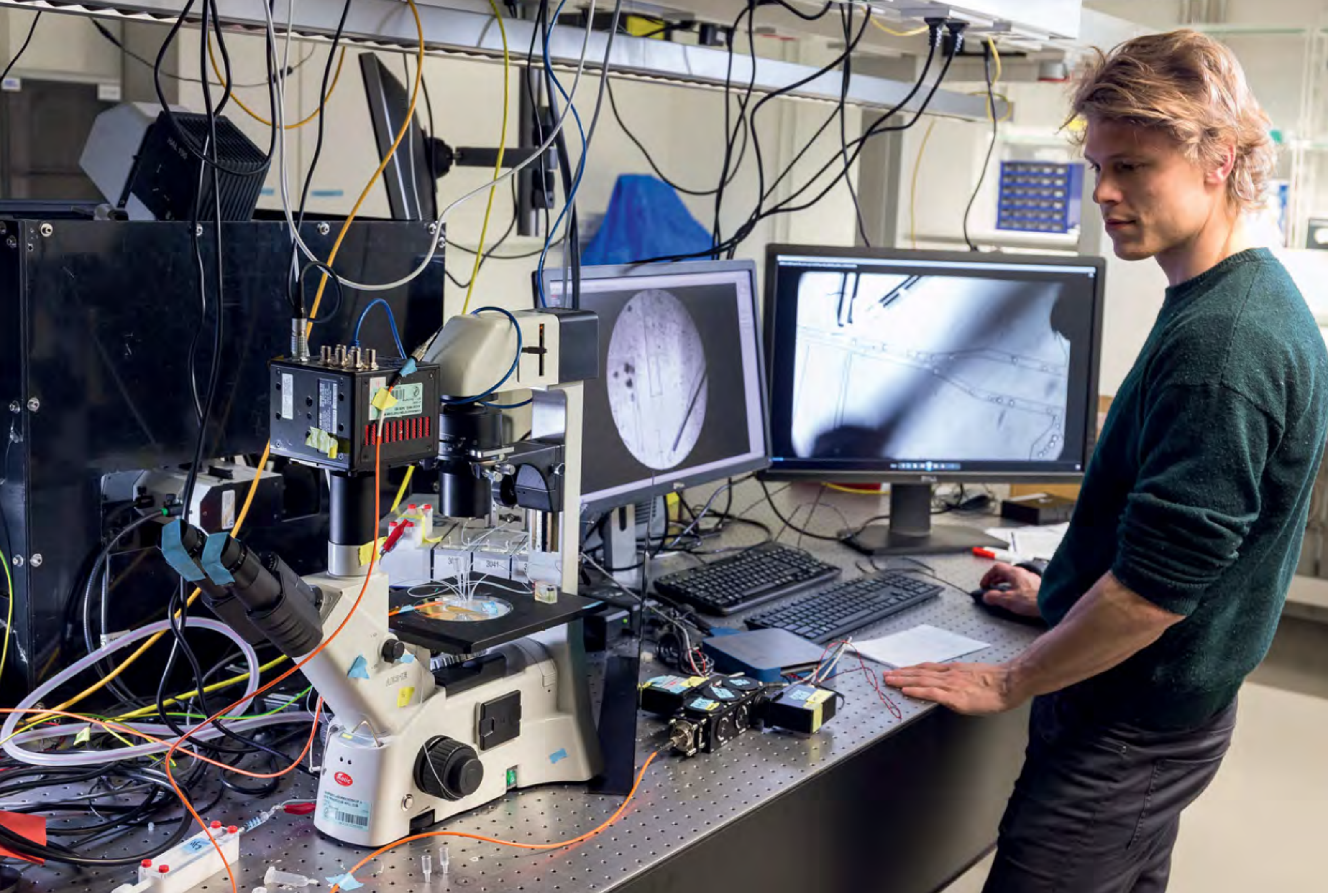
product: it may also have served as the repository for genetic material. The advantage of RNA: unlike DNA, it can fold more easily in three-dimensional form. This means that like modern proteins, RNA molecules form structures that act as natural catalysts and accelerate chemical reactions. Known as ribozymes, these RNA structures are not usually as efficient as protein enzymes, yet cells contain thousands of active ribozymes and they are central to the survival of every cell. The catalytic center of the ribosome, for example, consists entirely of RNA, i.e. it is a ribozyme. The ribosome may therefore even be a relic of a long-gone biological era, the so-called RNA world – a world in which RNA performed a dual function as a repository of information and a catalyst for chemical reactions.

But what triggered the formation of the longer RNA molecules from which the first ribozymes developed? In the Earth's early days, the individual building blocks, known as nucleotides, presumably bonded spontaneously to form very short RNA molecules. “Not much can be done with such short snippets; most of the RNA molecules found in cells are longer,” says Mutschler. However, for a long time it was unclear how the long chains needed for ribozymes could form. During the time he spent as a postdoc at the Laboratory of Molecular Biology in Cambridge, Mutschler discovered that in the right conditions, these snippets can themselves bond together to form RNA chains, even complex ones.

FREEZING AND THAWING

One particularly promising procedure, which is conducted several times in succession, involves freezing and thawing a saline solution containing RNA molecules. To do this, the researcher places short RNA chains designed at the computer into saline water. Then he cools the solution slowly. The liquid begins to freeze. At first, ice crystals consisting of almost pure water form, while the positively and negatively charged salt ions remain in the liquid. The fragile RNA likes this environment: the ions give it stability, help it to fold properly, and promote the bonding and linking of individual RNA fragments.

Mutschler then thaws the solution. The RNA strands, which were previously precisely arranged and held together by so-called hydrogen bonds, separate from each other and float about freely again. During the next freezing process, they rearrange themselves and form another bond. Mutschler has to repeat this procedure about twelve times until a strand consisting of around 200 building blocks is formed.



A microscope with image analysis software is able to recognize various cell types. This enables Kai Libicher to scan and sort 20,000 artificial model cells per second.

Photo: Axel Griesch

"This process would presumably happen by itself, but much too slowly. The repeated freezing and thawing accelerates the bonding considerably," explains Mutschler.

However, one long RNA strand is not yet enough to transfer information to the next generation. The RNA also has to be able to replicate itself independently. In modern biology, an enzyme called RNA polymerase helps it do this. However, this might not always have been the case. Mutschler is therefore currently investigating whether repeated freezing and thawing can also produce a ribozyme with a copy function.

Moreover, the temperature fluctuations have an additional effect: they appear to be able to trigger a basic mechanism that transfers genetic material between protocells. Tiny droplets of fat filled with genetic material adhere to each other during freezing and exchange their contents.

Mutschler's experiments in the laboratory have shown what repeated cycles of freezing and thawing can achieve. Now he has to prove that these processes could also have taken place in the environmental conditions that existed on Earth more than 3.5 billion years ago – the age of the oldest bacteria fossils yet known. Because conditions on Earth were quite chaotic during its early stages, the ecosystems that existed were highly diverse. "This is both a blessing and a curse," says Mutschler. Although this means that scientists can test a wide variety of environmental influences, the list of these is extremely long, ranging from deep-sea hydrothermal springs to craters left by meteorites.

Since many chemical reactions also require UV light, Mutschler assumes that life is more likely to have started at the surface of a body of water rather than deep in the ocean. He believes this most probably happened in hot springs located in cold environments, like those found in Iceland: "Simple molecules could easily have formed in the hot saline water of these springs and merged into more complex molecules in the cold surrounding areas."

PRIMORDIAL EARTH IN THE LAB

Mutschler and his colleagues now wish to investigate this hypothesis further by conducting experiments. To this end, they are cooperating with Paola Caselli's research group at the Max Planck Institute for Extraterrestrial Physics. This institution houses reaction chambers in which scientists can research the origin of organic compounds in space. However, they are equally capable of simulating the conditions that prevailed on the as yet unpopulated Earth. Environmental conditions such as temperature, light wavelength, and the

Hannes Mutschler is unable to turn back time, which is why he will probably never know with absolute certainty exactly what the first cells on Earth looked like. Yet for him, one thing is certain: RNA molecules played a central role in the emergence of life.

Photo: Axel Griesch

composition of the atmosphere can be set with great precision and varied as required. In this way, Mutschler aims to find out how stable RNA is under prebiotic conditions, and whether such conditions can support catalysis and evolution.

Scientists are increasingly coming to believe that genetic information, metabolism, and cell envelopes cannot give rise separately to living cells. Instead, it seems probable that all three of these elements evolved at the same time. The obvious assumption is that the components of life must have developed successively. However, the carefully configured reactions often do not work. They are only initiated when the reaction mixture contains exactly the right amounts of excipients and by-products. Consequently, it seems probable that a wide range of molecules came into being at more or less the same time.

Life may have therefore have emerged in a rather more disorderly fashion than in the researchers' test tubes. Mutschler also favors this idea. "I support the hypothesis that RNA performed a central role as a biocatalyst and an information repository when life first began. However, I also believe that RNA never acted alone and received help from other biomolecules right from the start."

If research into the origin of life is to be successful, it will therefore be crucial in the future to allow chaos in the test tubes – but only just as much as is necessary for life. ◂

www.mpg.de/podcasts/ursprung-des-lebens (in German)

SUMMARY

- Scientists want to know which components are essential for a cell to be viable. They are therefore attempting to reconstitute a cell with the minimum components – proteins, membrane molecules, and DNA or RNA.
- By repeatedly freezing and thawing solutions in the lab, researchers are able to link 200 building blocks for RNA molecules and form longer strands. The first longer RNA molecules on the primordial Earth could therefore have formed in hot springs located in a cold environment.
- The most important building blocks of life – genetic material, metabolism and cell envelope – may have come into being not successively, but more or less at the same time.

GLOSSARY

Coacervates: Aggregates of macromolecules held together by electrostatic forces between oppositely charged molecules. Chemical reactions can take place inside these structures, where they are protected from external influences; the structures range in size from a thousandth to a tenth of a millimeter.

Ribozymes: Like proteins (enzymes), certain RNA molecules are able to accelerate biochemical reactions. In addition to catalytically active RNA molecules, ribozymes of this type also comprise proteins to which catalytic RNA is bound. As catalysts, ribozymes reduce the activation energy of chemical reactions and thus accelerate them many times over. Ribozymes may have been the first self-replicating biological macromolecules on Earth, as they can function as information repositories and transmitters besides catalyzing chemical reactions.