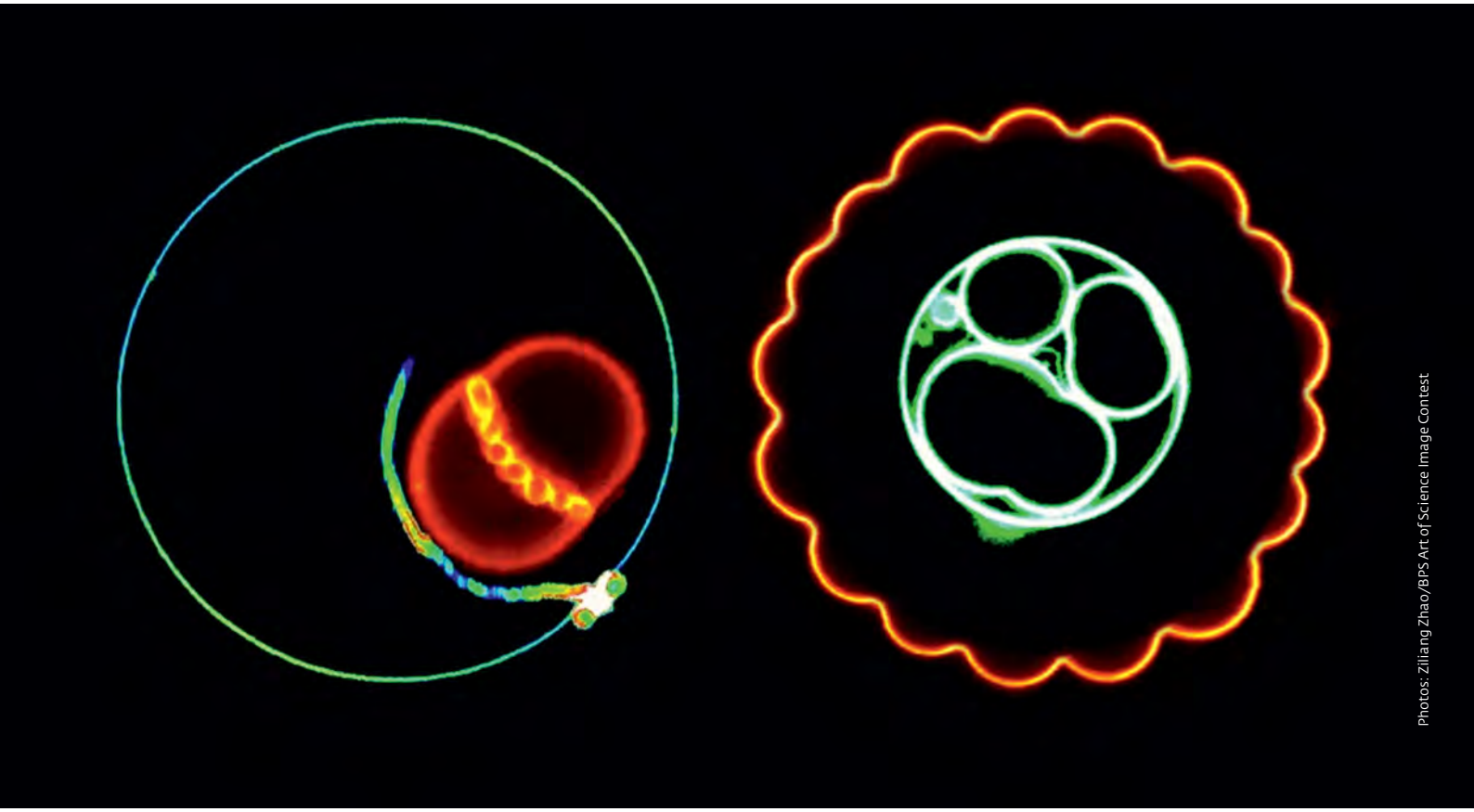
How cells **get their shape**

Some time around four billion years ago, life started to become encapsulated. The first cells emerged – protected spaces that facilitated the bonding of complex molecules. **Petra Schwille** from the **Max Planck Institute of Biochemistry** in Martinsried and **Rumiana Dimova** from the **Max Planck Institute of Colloids and Interfaces** in Potsdam are exploring the boundaries of cellular life. The two researchers are investigating the dynamics of biomembranes.



Photos: Ziliang Zhao/BPS Art of Science Image Contest

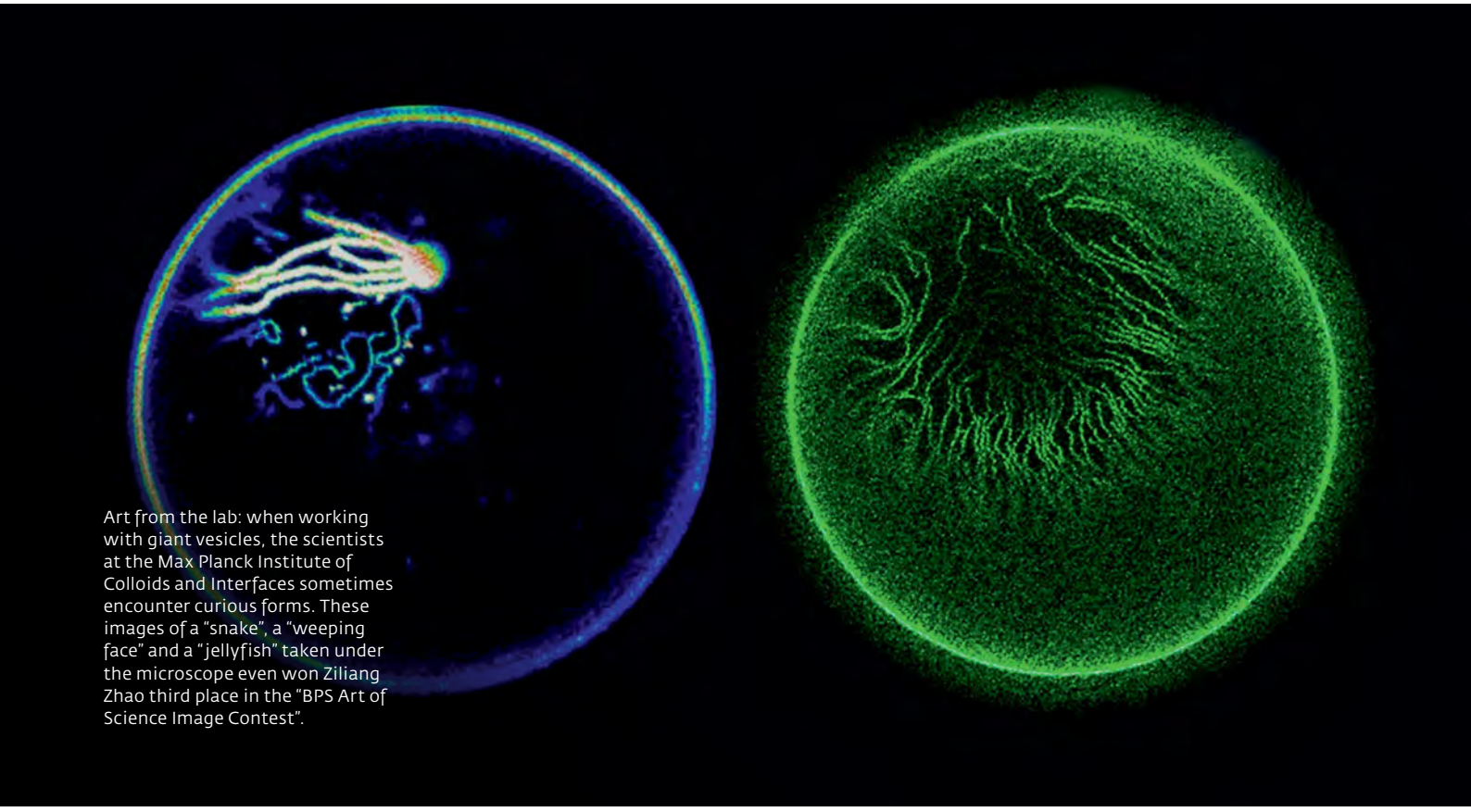
TEXT **ELKE MAIER**

Sometimes life becomes a matter for dispute – at least when we are talking about fossils that are three and a half billion years old. After an American palaeontologist discovered microscopic fossils in Western Australia in 1993, experts spent decades discussing whether these were the remains of living organisms or mineral structures that merely looked like cells.

These ancient relics reveal even less about how the first cells emerged from inanimate matter or about the principles according to which the earliest forms of life functioned. Neither does a look at the present-day living world offer much help: after billions of years of evolution, there are no organisms left on Earth that bear any resemblance to the very first lifeforms.

Researchers in the field of synthetic biology are therefore attempting to obtain new information about the functional principles of life in the laboratory. Nine Max Planck Institutes have joined forces to set up the MaxSynBio research network, which aims to create an artificial cell with minimal components as the lowest common denominator of life. The scientists involved are studying various aspects of living systems – energy supply, metabolism and movement, and growth and division.

"We are trying to break down the processes of life into individual modules and put them together to form a functioning system," says biophysicist Petra Schwille, Director at the Max Planck Institute of Biochemistry in Martinsried and coordinator of MaxSynBio. Like her colleague Rumiana Dimova at the Max Planck Institute of Colloids and Interfaces in Potsdam, she is investigating the mechanisms that



Art from the lab: when working with giant vesicles, the scientists at the Max Planck Institute of Colloids and Interfaces sometimes encounter curious forms. These images of a "snake", a "weeping face" and a "jellyfish" taken under the microscope even won Ziliang Zhao third place in the "BPS Art of Science Image Contest".

enable cells to divide and multiply. The researchers are focusing on the cell membrane – the thin membrane of liquid fat molecules that separates the cell from the outside and which made life possible in the first place.

Life, in fact, is the result of chemical alliances. Complex molecules such as proteins or nucleic acids can only form if countless individual building blocks are assembled in the right order. This is only possible if the molecular partners are available in sufficiently high concentrations to be able to join and bond. At the same time, these first delicate bonds are easily torn apart, especially in an aqueous environment.

A PROTECTED SPACE MAKES LIFE POSSIBLE

Therefore a closed space is needed – a space of the type provided by the first cells. "Cells are essential to life," says Petra Schwille. "It's inconceivable how life could exist without them." The crucial component is the cell envelope, which must be stable and afford sufficient protection while being flexible enough to facilitate growth and division – after all, life has to be able to reproduce.

The first protocells were probably simple, water-filled vesicles that consisted of fatty acids and contained self-replicating RNA molecules. In contrast, modern cells are surrounded by a plasma membrane consisting of phospholipids and embedded proteins. This membrane creates a protected space in which the countless chemical reactions that make up life take place; in addition, it facilitates the targeted transport of substances from the outside to the inside and vice versa. It is mechanically stable yet highly flexible so that the cell can grow and divide. "*Omnis cellula e cellula*" – all cells come from cells, wrote pathologist Rudolf Virchow in 1855.

But how do you make a cell divide in the lab? In such a way that it creates two viable daughter cells of equal size? This is the question being explored by Petra Schwille and her working group. The scientists aim to simulate this fundamental process with the aid of the so-called Min proteins that control cell division in the intestinal bacterium *Escherichia coli*.

The Department's clean room is opposite Schwille's office at the Max Planck Institute in Martinsried. Anyone who wants to go inside first has to step over a shoe rack containing a pair of plastic clogs. Located right behind the door, it blocks the way into the next lab, where no dust is allowed to enter. "This is where you take off your outdoor shoes," says Petra Schwille. "We put the shoe rack across the doorway so that nobody just walks in." This could easily happen to anyone lost in thought – and could possibly ruin the next experiment.

In the clean room, a scanning electron microscope and several micromanipulators are used to mill tiny chambers and ultra-fine channels in plastic

Photo: Axel Griesch

Left-hand page Petra Schwille and her team at the Max Planck Institute of Biochemistry study the behavior of cell division proteins in chambers and channels just a few thousandths of a millimeter in size. As even the tiniest dust particles could immediately clog the minute indentations, the researchers wear protective clothing and work in the clean room.

Right A thriving colony: Petra Schwille (right) and her doctoral student Beatrice Ramm are delighted that their *E. coli* cultures are flourishing. These bacteria serve as miniature factories from which the researchers obtain the cell division proteins they need for their experiments.

Photo: Axel Griesch

and silicone plates. In these indentations, which are just a few thousandths of a millimeter in size, the researchers study the behavior of the Min proteins under controlled conditions. Even the smallest dust particles would clog the fine structures immediately. "The Min proteins orchestrate cell division in *E. coli*," says Petra Schwille. "They show the cell where its center is located and consequently the right place to divide."

Inside the rod-shaped *E. coli* cell, the two proteins MinD and MinE flow back and forth between the cell poles. The driving force behind the incessant oscillation is the interplay between the two proteins: they form complexes, bind to the cell wall and are released shortly afterwards in response to certain biochemical signals. On their way through the cell, the Min proteins spend only a very short time in the middle. This results in a concentration gradient that directs another protein known as FtsZ to the center. "After the Min proteins have located the middle of the cell, FtsZ forms a central ring that initiates the actual process of division," the scientist explains. Without FtsZ, the cells would not be able to divide and would keep on growing longer.

PLENTY OF ACTION IN SPECIALLY DESIGNED CHAMBERS

The researchers in Martinsried obtain the cell division proteins for their experiments directly from *E. coli*. The microbes are cultivated in glass flasks and Petri dishes for this purpose. After isolating the proteins from the cells and purifying them, the scientists attach tiny fluorescent appendages that glow under UV light. This enables them to follow the movements of the proteins under the microscope as they happen.

To do this, they place the Min proteins in the specially made chambers and channels, which are first lined with thin layers of lipids in order to simulate conditions inside the cell. In 2013, Petra Schwille and her doctoral student at the time, Katja Zieske, were the first to succeed in making Min proteins oscillate outside a living cell using an artificial system of this kind. The molecules arrange themselves into artistic patterns, which the researchers can influence by changing the shape of the chambers. After adding the FtsZ protein, it is even possible to reproduce the first stage of the formation of a division ring in this artificial environment.

All this can now be facilitated in artificial cell envelopes. In order to make these, the researchers use the same phospholipids as those found in the plasma membrane of modern cells. Each of these molecules has a hydrophilic head containing a phosphate group and two hydrophobic tails consisting of long hydrocarbon chains. In order to accommodate these opposing preferences, the phospholipids arrange themselves in double layers, with the heads facing outwards and the tails inwards. This is the form they take in the

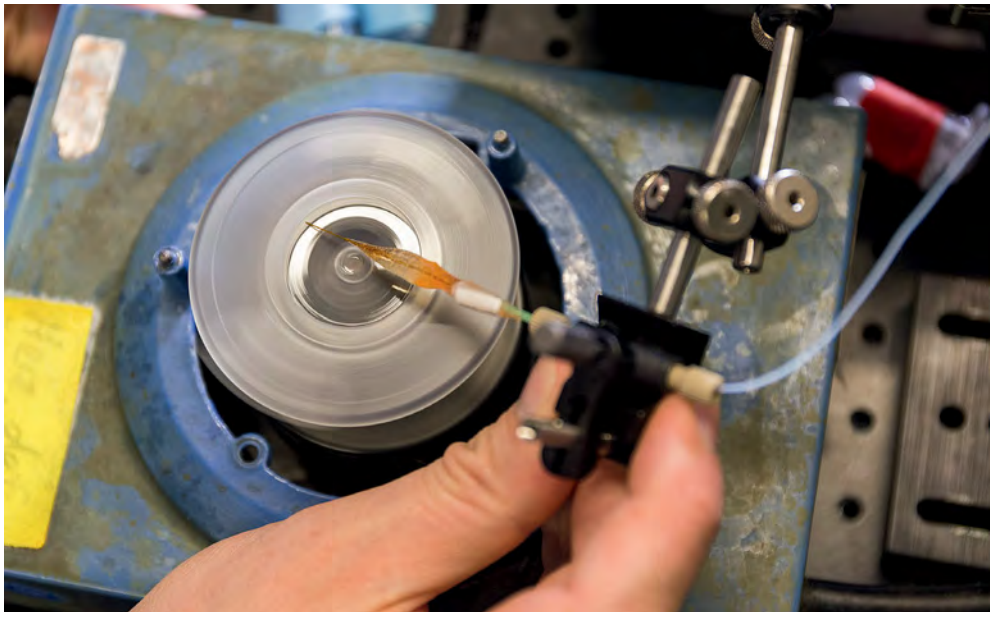
cell wall – a double layer only a few millionths of a millimeter thick.

Phospholipids readily form small bubbles in water/lipid mixtures. This process can be controlled by means of mechanical movement, e.g. centrifuging. This is how industrial companies produce liposomes as transport vehicles for cosmetics or drugs, and it is also the method used by the researchers in Martinsried to produce lipid bubbles as models for protocells. The starting point is a water/lipid mixture containing the cell division proteins encapsulated in the bubbles.

The Min proteins oscillate in these artificial cell envelopes as if they were in living cells, and the FtsZ division ring also forms. These oscillations, which occur solely as the result of self-organization, still fascinate Petra Schwille many years later. "Our goal for the next stage is to actually make the artificial cell divide," she says. Here the researchers are still looking for other factors that play a role in the division process.

Precision work: a special experimental set-up enables the researchers to encapsulate the Min proteins directly in the synthetic vesicles (bottom). Postdoc Michael Heymann performs this tricky task at the microscope with great patience and dexterity (top).

Photos: Axel Griesch (2)



Rumiana Dimova's work at the Max Planck Institute of Colloids and Interfaces in Potsdam has shown that in principle, the ability of cells to divide can be influenced by simple physical mechanisms. The Bulgarian biophysicist leads a working group in Reinhard Lipowsky's "Theory and Biosystems" Department.

She currently has even more work than usual, as she is also the editor and co-author of the mammoth book entitled "The Giant Vesicle Book", which will be published later this year. The "giants" (giant vesicles) are particularly large lipid bubbles up to 100 micrometers (a thousandth of a millimeter) in size. For Rumiana Dimova, they are the perfect model system: they are not only simple to produce and handle but can also be manipulated and observed easily on account of their size. This enables researchers to see directly under the microscope how the cell membrane reacts to certain chemical substances or electrical impulses, for example.

In order to obtain the giant vesicles, Dimova and her team usually use ready-made phospholipids and solutions available from laboratory supplies. "This is very convenient, although the resulting model system is drastically simplified," says the scientist. If specific experiments require vesicles that approximate as closely as possible to natural ones, the researchers use living cells and expose them to a mixture of chemicals.

This chemical cocktail stimulates the cells to form so-called "blebs", tiny protrusions in the cell wall that grow in size and ultimately separate from the cell in the form of giant vesicles. As these contain the same substances as the cells from which they originated, they are particularly useful as realistic models. The researchers in Potsdam use them to find out how physical factors affect the form, mechanical attributes, and growth of the cell envelope along with its ability to divide. For this, they turn their attention to the interior of the giant vesicle.

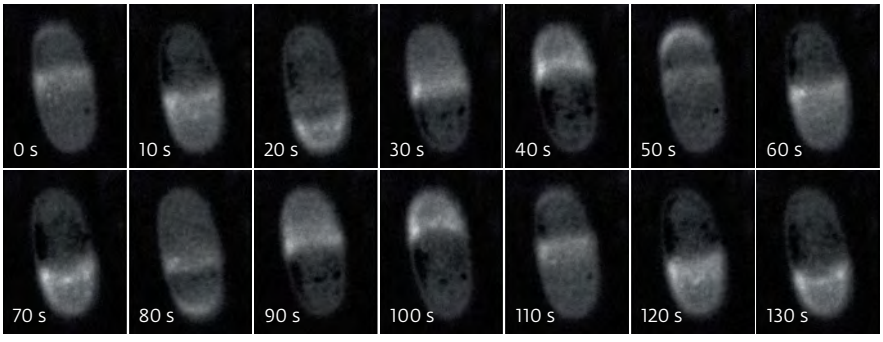
DROPS OF OIL IN VINAIGRETTE DRESSING

A living cell houses various organelles, each of which performs different functions. The Golgi body, for example, secretes proteins, while the mitochondria provide a supply of energy. These classic organelles are enveloped in one or even two membranes. However, the cell also contains organelles with no membranes, compartments with no fixed boundaries that most resemble drops of oil in vinaigrette dressing. Proteins or ribonucleic acids (RNA), for example, are concentrated in these reaction chambers with no fixed confines so that specific reactions can occur.

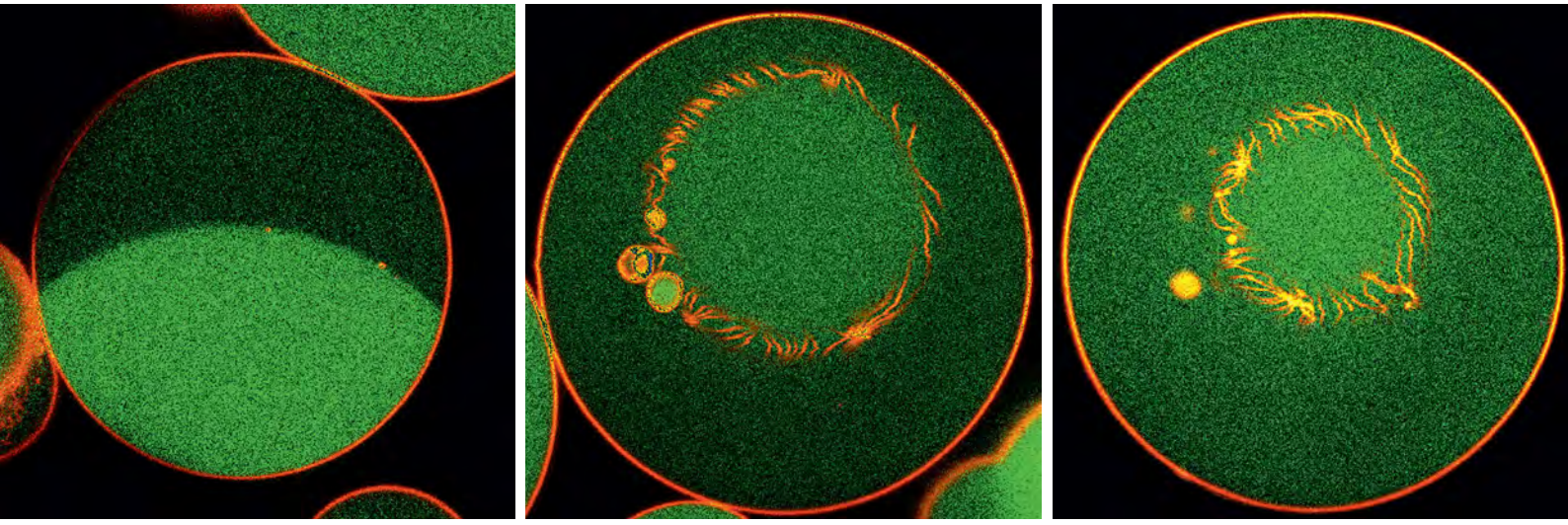
The most well-known of these membraneless organelles is probably the nucleolus inside the cell nucleus, in which components for ribosomes are produced. These nucleoli were first described back in the 1830s. Since then, researchers have identified many such liquid compartments. Some of them exist for just a short time and then disintegrate. They are found in the cell nucleus, cell plasma, or right on the inside of the cell envelope. "Membraneless organelles are currently one of the hot topics in biophysics," says Rumiana Dimova.

How do such liquid structures develop in the liquid interior of the cell, and how do they manage to keep their shape? What happens when these inclusions come into contact with the cell membrane? And how does this influence the cell's shape? Little time has been spent on investigating these and similar questions to date, but research in this area is rapidly gathering momentum. >

Constant oscillation: cell division proteins MinD and MinE display the same behavior in synthetic chambers as in living cells. They oscillate from pole to pole and generate concentration gradients that show where the center of the cell is located.



Photos: Angew Chemie Int Ed. 52 (1), 459–462 (2013)



The scientists in Potsdam are using giant vesicles as chambers in which to simulate the formation of these membraneless compartments. "The mechanism behind this is phase separation," says Rumiana Dimova – the same mechanism that causes oil droplets to form in a well-mixed vinaigrette dressing as soon as it is left to stand. In order to create a two-phase system, the researchers let the vesicles grow in a solution to which two water-soluble polymers, polyethylene glycol (PEG) and dextran, have been added.

"The polymers become encapsulated in the giant vesicles," explains the scientist. "We then increase the osmotic concentration of the surrounding medium. This causes the water in the vesicles to penetrate the membrane to the outside, as a result of which the concentration inside the vesicle increases. The increased polymer concentration inside the vesicles causes two phases to separate: two droplets form and can be easily viewed under the microscope."

Rumiana Dimova and her team are investigating how these droplets interact with the cell membrane. What happens when one of these droplets touches the membrane? Does it merely touch the membrane, or does it also wet it? "This has a decisive influence on the dynamics of the membrane, for example its curvature," says the scientist.

A BALLOON LEAKING AIR

This type of two-phase system even allows researchers to reconstruct the composition of membraneless organelles. For this, they let their vesicles grow in a solution that contains biopolymers such as proteins and RNA instead of PEG and dextran. "Proteins and RNA are the main components of membraneless organelles," says Dimova. "And there are plenty of possible points of contact with membranes inside the cell – not only on the inside of the cell envelope, but also on the endoplasmic reticulum, for example." This is a densely branched channel system enclosed by membrane that accounts for more than half of all the membranes inside the cell.

Moreover, the giant vesicles can be made to divide – just by continuing to increase the osmotic concentration outside the vesicle. This causes the pressure inside the vesicle to drop so far that the droplets drift apart until they are touching the vesicle's inside wall. By this stage, there is no tension left in the vesicle wall – like a balloon from which all the air has leaked. Droplets wetting the membrane can cause the membrane to bulge and constrict the vesicles.

"Our experiments have shown that simple physical processes such as phase separation and wetting have an enormous influence on the shape of cells and their organelles," says Rumiana Dimova. Depending on whether the nearby membrane is curved or under tension, the envelope can protrude inwards as well as outwards. "This type of invagination facilitates the flexible storage of cell wall material that is not currently needed," says the scientist. Structures of this kind may even have played a role in evolution as the precursors of organelles enclosed in membrane such as the endoplasmic reticulum.

Yet why is cell division in nature so complicated when it could be so much easier? Why did evolution come up with something as complicated as the Min system in *E. coli*? "'Why' questions are even harder to answer in the field of biology than in the other natural sciences," says Petra Schwille. "There isn't actually any objective reason why this type of cell division should be better

Photos: Rumiana Dimova/MPI of Colloids and Interfaces

Left-hand page The researchers in Potsdam use water-soluble polymers to generate a two-phase system inside the giant vesicle similar to a drop of oil in a vinaigrette dressing. If one of these droplets touches the vesicle wall, the wall can deform, protrude inwards and even form tubular structures (second and third images from left).

Right Rumiana Dimova and her colleague Ziliang Zhao at the Max Planck Institute of Colloids and Interfaces view the results of their two-phase experiments at the computer.

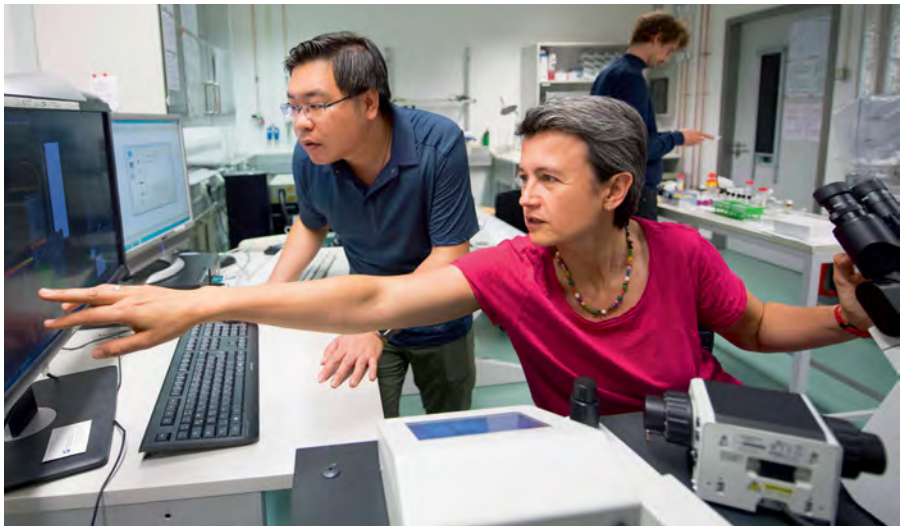


Photo: Jens Jeske

than all the others. However, it's ideal for the rod-shaped bacteria, and the oscillations presumably have ancillary effects – they might help distribute the DNA evenly in the daughter cells, for example. This isn't certain though."

What is certain, however, is that the separation of the genetic material and the actual division of the cell are a key stage on the way to new life. Both processes have to be perfectly coordinated if two viable daughter cells are to result. This precise spatial and temporal coordination requires a sophisticated system.

Yet will it ever be possible to find out how the first cells isolated themselves from their environment and what caused these cells to start dividing? How can we know what actually happened when matter made the transition from inanimate to animate? Petra Schwille takes a pragmatic view of these concerns: "We don't necessarily have to become fixated on how the first cells worked in order to understand the fundamental principles of life," she wrote in an essay. "Instead, we should concentrate on the basic modules of living systems. After all, the first functional flying machines built by humans were not made of feathers." ◂

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SUMMARY

- **The MaxSynBio research network was established jointly by nine Max Planck Institutes with the aim of creating a synthetic cell with the minimum components necessary. In this context, the scientists are focusing on the basic mechanisms of living systems such as growth and division.**
- **Cell division in the intestinal bacterium *Escherichia coli* is controlled by so-called Min proteins. These form concentration gradients inside the cell that show where the new cell wall is to be drawn in.**
- **Even simple physical mechanisms such as phase separation and wetting have an enormous influence on the cell's shape, mechanical properties, growth and ability to divide.**

GLOSSARY

Organelle: Definable area inside a cell to which a specific function can be assigned.

Plasma membrane: Biomembrane that encloses modern-day cells. It separates the cell from the outside world while facilitating exchange; it also performs many other functions.

Protocells: Precursors of the first cells. The first lifeforms are believed to have originated from these around four billion years ago.

Ribonucleic acid (RNA): Single-strand macromolecule consisting of four different nitrogenous bases, a ribose sugar and a phosphate group. RNA performs a variety of tasks inside the cell. Among other things, it is responsible for transmitting the protein building instructions from the DNA in the cell nucleus to the ribosomes.

Wetting: Describes the behavior of liquids when they come into contact with solid or fluid bodies – in this case the lipid membrane. Wetting influences the physical properties of the membrane and thus affects its shape and ability to divide.