



Algae, like most higher plants, use the enzyme RuBisCO to fix carbon dioxide. RuBisCO is the most commonly used biocatalyst for this purpose, but it isn't the most efficient. Scientists are therefore experimenting with other enzymes and metabolic processes to more efficiently convert carbon dioxide into organic molecules.

# Metabolism 2.0

Over 50 million genes and 40,000 proteins: combing through international databases for likely candidates, **Tobias Erb** and his colleagues at the **Max Planck Institute for Terrestrial Microbiology** in Marburg were faced with an overwhelming choice. In the end, the scientists picked out just 17 enzymes for the first synthetic metabolic pathway that is able to convert carbon dioxide into other organic molecules. Now they have to show that the cycle they sketched out on the drawing board also works in living cells.

TEXT **KLAUS WILHELM**

It sounds almost too good to be true: a means of counteracting the greenhouse effect, removing excess carbon dioxide from the atmosphere and turning it into environmentally friendly products. Carbon dioxide levels have risen by around 30 percent during the past 100 years, contributing greatly to global warming. A method that removes excess carbon dioxide from the atmosphere while also serving practical purposes would thus be extremely welcome.

Nevertheless, Tobias Erb's primary aim isn't the fight against climate change. The researcher first wants to understand how gaseous carbon dioxide can be converted into organic molecules. "Of course, if we could exploit the greenhouse gas as a carbon source using biological methods and remove it from the atmosphere in the process, that would be a great side benefit," says the Max Planck researcher.

Erb studied biology and chemistry, and even at an early age was fascinated by the question of what makes life tick down to the smallest scale. "I've

always been interested in how microscopic life forms, such as bacteria, do things that chemists can still only dream about," he says. Erb spent the first years of his research career studying bacterial enzymes – protein biocatalysts that initiate, accelerate or halt chemical reactions.

## ALTERNATIVE TO RUBISCO

In his doctoral thesis, Tobias Erb turned his attention to the carbon cycle, the process by which atmospheric carbon dioxide is converted into various sugar compounds. In a purple bacterium, he discovered an enzyme with the unwieldy name crotonyl-CoA carboxylase/reductase (CCR). This introduces carbon dioxide molecules into the bacterium's metabolism.

Besides bacteria, plants are the main users of this process, known as carbon dioxide fixation. During photosynthesis, plants harness sunlight as an energy source to produce sugar from atmospheric carbon dioxide. To do this, they use a metabolic pathway known as the

Calvin cycle, which is described in every biology textbook along with all the enzymes involved. The Calvin cycle is essential for life on Earth, as plants use it to produce vital organic molecules, such as sugars, for other life forms.

For a long time, the Calvin cycle was believed to be the only pathway for carbon dioxide fixation. "But we've since discovered a good half dozen more," Erb explains. "More than a third of the carbon dioxide on this planet is bound by microorganisms." Nature has thus devised various solutions to the same problem. They all work, but none is perfect.

One example is the carbon-dioxide-fixing enzyme in the Calvin cycle called RuBisCO. Erb describes it as "the most underestimated enzyme on our planet because it's the most common." For every person on Earth, there are around five kilograms of RuBisCO in the biosphere. The enzyme is able to produce a pinch of sugar from the carbon dioxide contained in the volume of a normal living room. Nevertheless, RuBisCO works relatively slowly and



**Left page** A specific enzyme is responsible for every reaction in the CETCH cycle. Crotonyl-CoA carboxylase/reductase (CCR), for example, facilitates the two carbon-dioxide-fixing reactions.

**This page, top** Together with Tobias Erb, Nina Cortina is investigating the CETCH cycle. The Philippine scientist is an expert in mass spectrometry. In the foreground is a photospectrometer for carrying out measurements on enzymes.

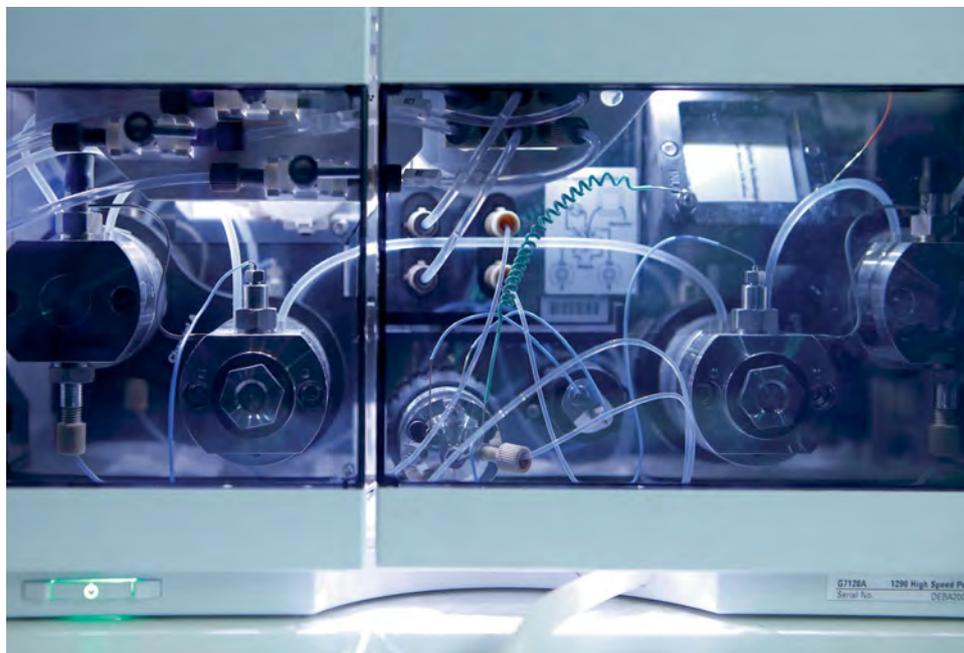
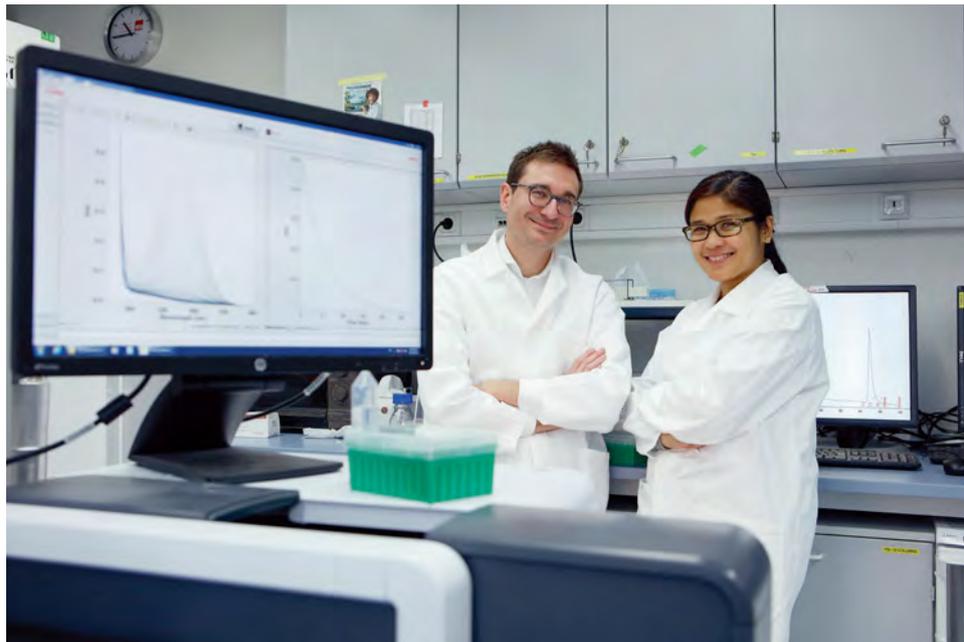
**This page, bottom** The researchers use such mass spectrometry instruments to analyze the reaction products of the CETCH cycle.

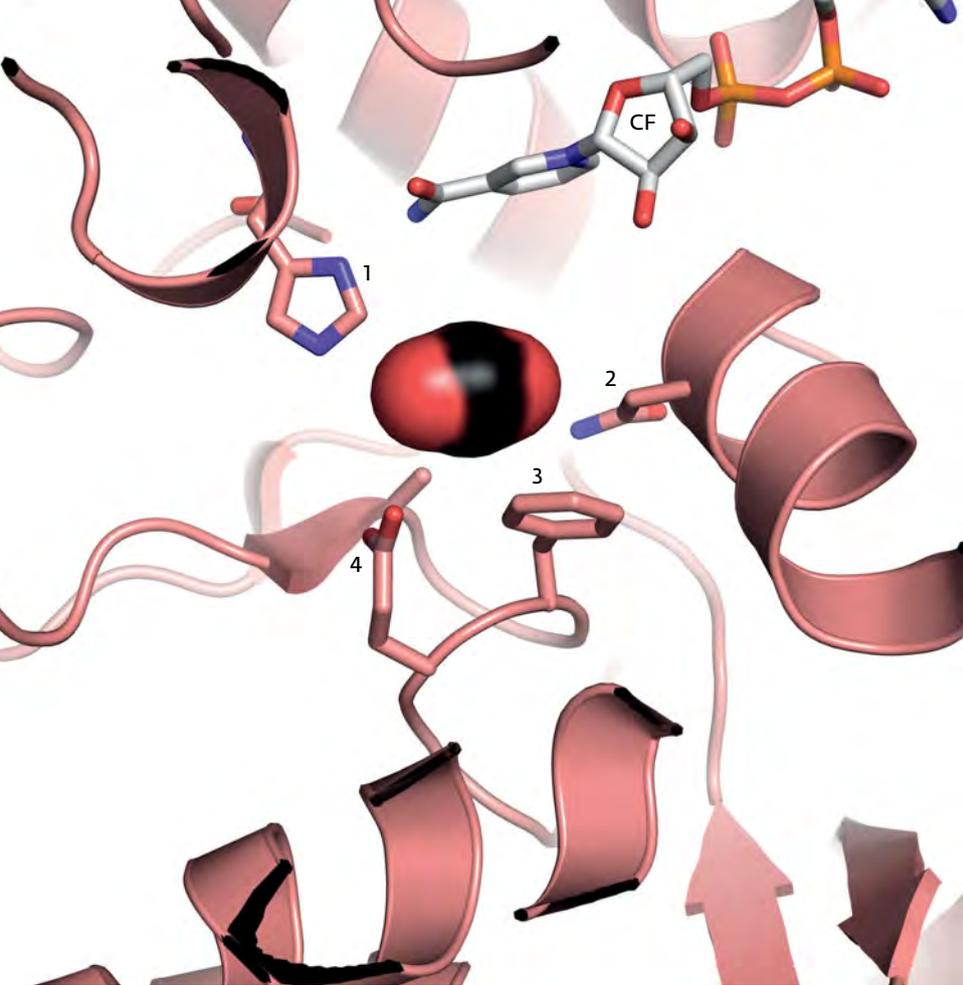
form a completely new metabolic pathway for carbon dioxide fixation.

In 2011, Tobias Erb – then at ETH Zurich – outlined the cycle, called CETCH, complete with all the relevant biochemical reactions, in just two weeks. In addition to drawing on his knowledge of carbon dioxide metabolism, he consulted international databases listing more than 50 million genes and more than 40,000 enzymes and their respective functions.

From those, Erb selected several dozen candidates that, together with the turbocharged CCR enzyme, could perform the desired functions in his artificial cycle: “After studying the natural process of carbon dioxide fixation for so long, I was convinced that our designer pathway could also be realized in practice.”

Even before moving from Zurich to the Max Planck Institute for Terrestrial Microbiology in Marburg, Erb set up a team “without hierarchies and with talented researchers who want to push scientific boundaries.” Applying passion and consummate expertise, they trans-





**Left** The active center of the CCR enzyme (pink lines and bands; CF: cofactor NADPH): The position of the egg-shaped carbon dioxide molecule in the center was modeled on a computer. The amino acids important for positioning the carbon dioxide molecule are shown enlarged: histidine (1), asparagine (2), phenylalanine (3) and glutamate (4).

**Right** Erb first designed the CETCH cycle on the computer and later tested it with his team in the lab. The result: the first artificial carbon-fixing metabolic pathway.

lated their model from the drawing board into reality in a record time of just two years.

The scientists tested the functionality of new enzyme candidates, modified them and tried out new combinations until they worked optimally together. "Despite all the laboratory technology, this still involved a lot of manual work," says Thomas Schwander. "Time and again we had to overcome new hurdles." For a long time the researchers were unable to get the cycle going because one of the enzymes only worked with an iron compound, which, however, caused the other proteins to flocculate. The enzyme therefore first had to be modified so that it could work with the more suitable substrate oxygen.

Another difficulty lay in that fact that the cycle was initially plagued by numerous unwanted side reactions. As a result, it was slow and tended to grind to a halt quickly. It was only when the scientists added other enzymes to the original design that they were able to eliminate the unwanted reactions. These additional enzymes acted as recycling forces to correct the errors of

the other enzymes. Tobias Erb suspects that such corrective loops may also play an important role in natural metabolic pathways.

Despite all the difficulties, the researchers ultimately succeeded in cobbling together the first man-made metabolic pathway for carbon dioxide fixation. It involves 17 enzymes from nine different organisms and includes three designer enzymes that the scientists modified from existing enzymes with the help of a computer so that they work more precisely or catalyze other reactions.

#### RAW MATERIALS ON TAP

The enzymes are therefore natural in origin, but their combination to form a novel, highly efficient metabolic pathway doesn't occur in nature. "Presumably, the enzymes never had the chance to come together in nature in the course of evolution," Schwander says. Erb's carbon dioxide cycle culminates in the formation of a compound called glyoxylic acid. However, the cycle could be modified to produce raw

materials for biodiesel or other organic substances instead.

Carbon dioxide fixation requires energy. The CETCH cycle is driven by chemical energy or, more specifically, by electrons. The Calvin cycle of photosynthesis works with solar energy, which is then converted into chemical energy. The researchers were therefore able to compare the two processes to determine which is more efficient. Whereas the CETCH cycle consumes only 24 to 28 light quanta to bind a carbon dioxide molecule, natural photosynthesis takes 34. "So we could fix about 20 percent more carbon dioxide with the same amount of light energy," Erb points out.

And that's not even the upper limit. Erb's team is already working on developing even thriftier carbon dioxide cycles. In the future, these synthetic fixation cycles might be coupled with solar cells. The electrons the solar cells produce from sunlight could be used to convert carbon dioxide into other compounds. Such visions no longer appear technically unfeasible. For example, researchers in the MaxSynBio network



are working intensively on processes at the interface between chemistry, materials science and biology.

In the context of synthetic biology, the CETCH cycle could also help to improve natural photosynthesis. However, the genes for the enzymes involved in the CETCH cycle would first have to be inserted into a living cell – a bacterium, an alga or a plant – which would then synthesize the desired product.

In the next step, the Marburg-based scientists want to engineer bacteria to use the CETCH genes as intended. “We can’t predict how our cycle of 17 reactions will behave in a cell in which 3,000 reactions of all kinds are taking place. We still have a few more years of work ahead of us,” says Erb.

The biomodule of the CETCH cycle may eventually end up in Craig Venter’s minimalist cell – or even better, in an artificial cell to be created by the MaxSynBio network. In any case, it will still take some time for Erb’s dream of “creating an artificial metabolism 2.0 that is able to produce any desired organic compound from carbon dioxide” to become a reality. ◀

### TO THE POINT

- The carbon-dioxide-fixing plant enzyme RuBisCO works slowly and frequently makes errors. By comparison, the bacterial enzyme crotonyl-CoA carboxylase/reductase (CCR) is around 20 times faster and more accurate.
- Together with 16 other enzymes, the CCR enzyme can be added to a test tube to produce the CETCH metabolic pathway. The artificial cycle converts carbon dioxide more efficiently than the Calvin cycle used by plants.
- Bacteria and plants could one day use the CETCH cycle to fix excess atmospheric carbon dioxide and convert it to useful organic substances.

### GLOSSARY

**Calvin cycle:** A metabolic pathway in plants in which atmospheric carbon dioxide is converted into sugar molecules. The cycle uses ATP as an energy source. The enzyme RuBisCO makes it possible to convert carbon dioxide to the sugar ribulose-1,5-bisphosphate (carbon dioxide fixation). The cycle must run three times, fixing three carbon dioxide molecules to produce one molecule of the sugar. The chemical energy required for the Calvin cycle is obtained from the light reaction of photosynthesis.

**Synthetic biology:** A young research field whose aim is to develop biological systems that don’t occur in nature. A first step is the construction of the simplest possible cell whose genome contains only information that is absolutely essential for the cell’s survival. There are two opposing approaches: some researchers try to reduce the complexity of existing cells to the bare minimum (top-down approach), while others aim to identify the building blocks that are absolutely vital for the cell’s survival and use them to construct new cells (bottom-up approach). The objective is to simplify existing biotechnological processes, but completely new products such as vaccines, medicines, diagnostic aids, biofuels and tailor-made materials could also be designed with the help of synthetic biology.