Scientists provide new insights into the citric acid cycle

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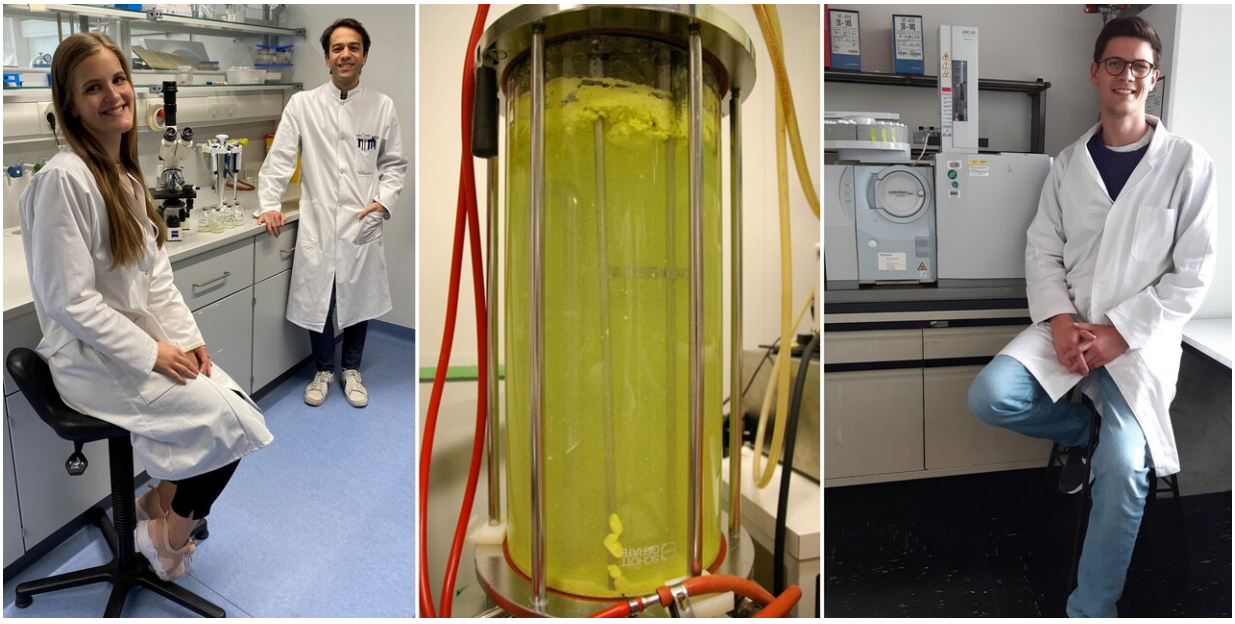

Lydia Steffens and Eugenio Pettinato (University of Münster, left) and Thomas M. Steiner (TUM, right) in the laboratory; the three doctoral students share first authorship of the Nature publication. In the middle: a fermenter system for growing bacteria. Credit: Informationsdienst Wissenschaft e. V.

Researchers led by Professors Ivan Berg (University of Münster) and Wolfgang Eisenreich (TUM) report new insights into the citric acid cycle: Certain bacteria can use this central metabolic pathway "backward," but to do so, they must have very high concentrations of the

enzyme citrate synthase and of carbon dioxide. This pathway may be a relic from the early development of life.

The citric acid cycle is an important metabolic pathway that enables living organisms to generate energy by degrading organic compounds into carbon dioxide (CO_2). The first step in the cycle is usually performed by the enzyme citrate synthase, which builds citrate. But in the absence of oxygen (under anaerobic conditions), some bacteria can perform the reverse cycle: They can build up biomass from CO_2.

In this so-called reversed citric acid cycle, citrate synthase is replaced by ATP-citrate lyase, which consumes cells' universal energy carrier ATP (adenosine triphosphate) to cleave citrate instead of forming it. However, a few years ago, a research team led by Ivan Berg (University of Münster) and Wolfgang Eisenreich (Technical University of Munich) discovered that instead of requiring ATP-citrate lyase for the reversed cycle, some anaerobic bacteria can use citrate synthase itself to catalyze citrate cleavage without consuming ATP. Now, the same team found that bacteria using this metabolic pathway (the reversed citric acid cycle through citrate synthase), depend on very high concentrations of both the enzyme and carbon dioxide.

As a comparison, the CO_2 concentration in air is around 0.04%, but bacteria using this pathway require at least 100 times more than that for their growth. The researchers assume that such CO_2 concentration-dependent pathways could have been widespread on the primordial earth, since the CO_2 concentration was high at the time. Therefore, this metabolic pathway may be a relic of early life. The results of the study have been published in the journal *Nature*.

The team studied the anaerobic bacteria Hippea maritima and Desulfurella acetivorans. These organisms live without oxygen in hot springs, where the CO_2 concentration can be 90% and higher. "It is

conceivable that many other organisms use this cycle to bind CO_2," says Ivan Berg. "Our findings are in line with the results of several recent studies highlighting a potential widespread occurrence of this reversed oxidative citric acid cycle."

Nevertheless, many bacteria use the energetically less efficient ATP-dependent reaction for citrate cleavage. "It was mysterious why this 'expensive' version of the pathway exists if an energetically much cheaper alternative through the backward reaction of citrate synthase is feasible. Now we know that this is due to the low CO_2 concentrations in many environments. The cheap alternative doesn't work there," says Wolfgang Eisenreich.

These findings could also be of interest for biotechnology. With the knowledge that autotrophic organisms using this "backward cycle" depend on the CO_2 concentration, scientists can apply it to more efficiently convert substrates into value-added products.

The results in detail

The scientists wanted to understand what factor determines whether the citric acid cycle runs "forward" or "backward" in the bacteria. Cultivating the bacteria under different conditions, they noticed that the growth of these organisms was highly dependent on the CO_2 concentration in the gas phase. In detail, the high CO_2 concentration was needed to allow the function of another important enzyme, pyruvate synthase. This enzyme is responsible for assembling acetyl coenzyme A (acetyl-CoA), the product of the "reversed cycle."

The high CO_2 concentration drives the pyruvate synthase reaction in the direction of carboxylation and the entire cycle backward, enabling CO_2 to be converted into biomass. The studied Hippea maritima and Desulfurella acetivorans were able to grow very well at 20% and 40%

CO_2 in the gas phase but only moderately at 5% CO_2, and no growth was possible at 2% or 1% CO_2. As a control, the scientists studied another autotrophic bacterium Desulfobacter hydrogenophilus, which uses the energetically more expensive ATP-citrate lyase version of the reversed citric acid cycle. In this bacterium, growth was not affected by the CO_2 concentration.

The "backward cycle" that uses citrate synthase for citrate cleavage cannot be bioinformatically predicted, as it does not have the key enzymes whose presence can be used as a marker for the functioning of the pathway. Therefore, as an identifying feature for bioinformatic analyzes, the scientists used the detected high levels of citrate synthase in these bacteria's protein cocktail. Using a special analysis tool, the researchers were able to predict the production levels of individual proteins. With this trick, it was possible to predict the functioning of the "backward cycle" for inorganic carbon fixation in many anaerobic bacteria.

The scientists also showed that no gene regulation was necessary for switching from the oxidative ("forward") to the reductive ("reverse") direction. "This means that the cells can react very quickly on the availability of the carbon source in the environment" says Ivan Berg. "They use either the reductive direction to fix CO_2, if the concentration of CO_2 is high, or the oxidative direction, if another carbon source is available."

Methodological notes

The methods used in the study were mass spectrometry and 13C-isotope analyses, enzyme measurements, protein quantification as well as media and amino acid analyses using chromatographic and spectrometric methods (LC/MS or GC/MS). With bioinformatic methods, they examined the occurrence of certain nucleotide base combinations

(codons) in order to make predictions about the production of individual proteins.

More information: Lydia Steffens et al. High CO_2 levels drive the TCA cycle backwards towards autotrophy, *Nature* (2021). DOI: 10.1038/s41586-021-03456-9

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