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How microorganisms gather together a metabolic pathway

Many microorganisms are found in remote places in conditions of extreme heat, extreme cold or in areas with high salt concentrations. The majority of microorganisms feed on low-molecular carbon compounds, which gives rise to the question as to how they manage to turn these compounds into cell building blocks. In a recent publication in the renowned journal Science, Dr. Ivan Berg and his group of researchers at the University of Freiburg report on the possibility that Dead Sea microorganisms produce amino acid precursors. This finding also provides new insights into evolutionary tools as it can be assumed that the microbes did not develop this talent on their own.

The generation of energy, the biosynthesis of amino acids and the assembly of carbohydrates are all processes that are prerequisites for life on earth. Microorganisms in particular have developed a variety of metabolic pathways in order to ensure their survival in extreme environments. Dr. Ivan Berg and his group of researchers at the Department of Microbiology of the Institute of Biology II at the University of Freiburg are looking for unknown metabolic pathways in microorganisms.

"I am interested in how microbes are able to turn small carbon compounds into more complex ones that are used as cell building blocks," said Berg. A microbe from the group of salt-tolerant archaea, which are among the most primordial life forms on earth that have managed to survive in extreme environments, proved to be a rich source for their investigations. The microorganism in question, Haloarcula marismortui, uses a previously unknown strategy to turn acetate (salt of acetic acid; consists of two carbon molecules) into a four-atom molecule and makes it suitable for the biosynthesis of amino acids.

A third variant

Dr. Ivan Berg standing in front of a tank used to culture microorganisms.

Acetate is mainly used by organisms as acetyl coenzyme A (CoA), which is an important molecular node in the network of cellular substance cycles. Acetyl-CoA is an important molecule in many biochemical reactions; it is used to create fatty acids and is hence the starting substance for the storage and generation of energy. Indirectly, it is also involved in the synthesis of amino acids and is

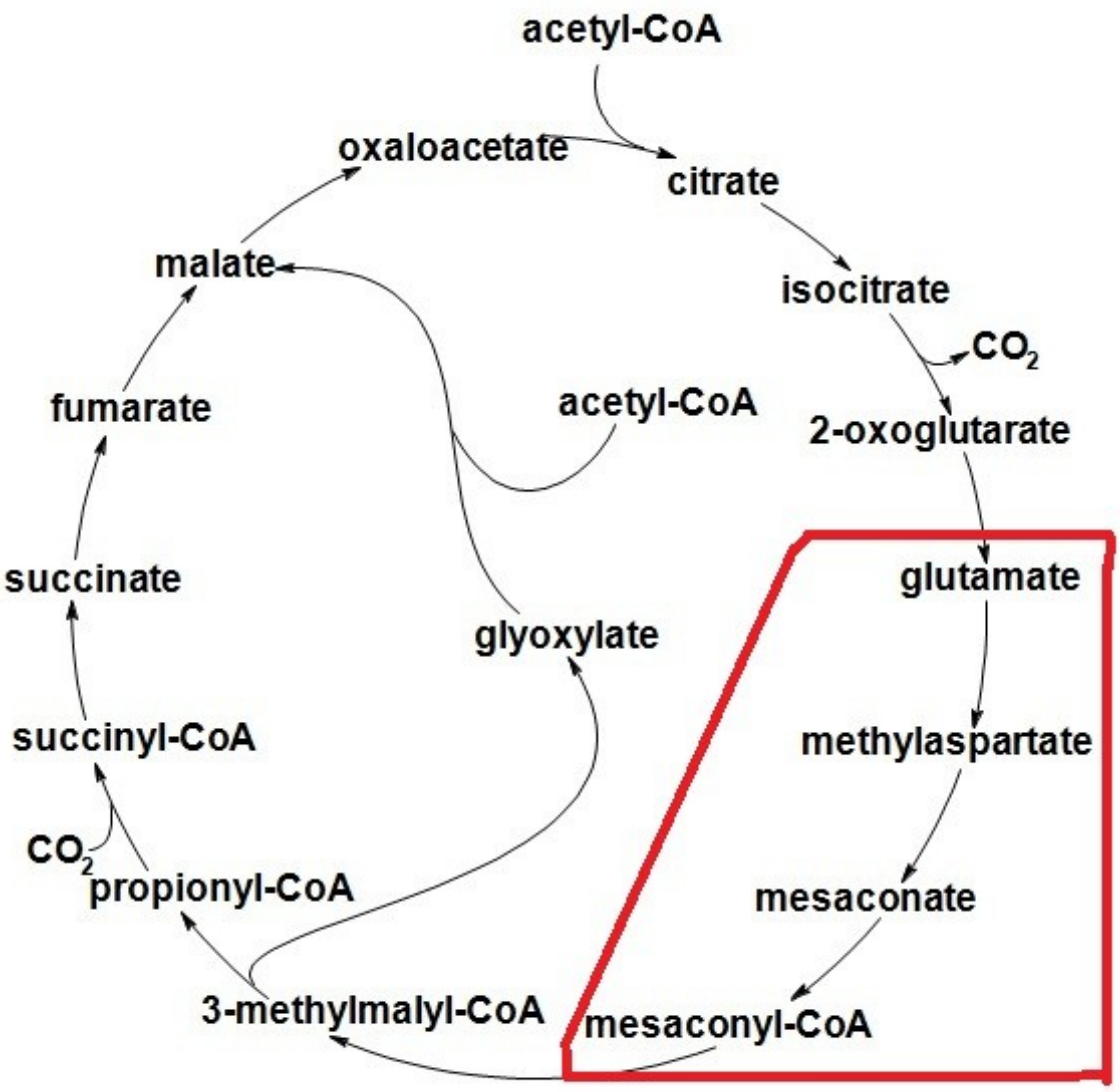
hence essential for the synthesis of proteins. Microorganisms that live in the highly salty conditions of the Dead Sea cannot take up amino acids with their food. Instead, they have to produce them from low-molecular compounds. However, amino acids can only be produced from molecules such as malate or succinate, which have a carbon backbone consisting of four atoms. Many organisms lack the enzymes required to metabolise acetate directly. "The microbes are therefore faced with the problem of how to turn a C2 molecule into a C4 molecule," said Berg. Many substrates enter the central carbon metabolism via acetyl-CoA. Up until Berg's discovery, only two such pathways, the glyoxylate cycle and the ethylmalonyl-CoA pathway, were known. It appears that Haloarcula marismortui has developed a third variant by which acetyl-CoA is oxidised to glyoxylate via the key intermediate methylaspartate. The researchers from Freiburg named the new metabolic pathway "methylaspartate cycle" after its key intermediate.

What is the best way to discover a new metabolic pathway? Berg and his team have chosen the microorganism Haloarcula marismortui for their investigations as it was known for its ability to grow in media with acetate as the only source of nourishment. Initial investigations revealed that H. marismortui lacked the enzymes of the glyoxylate cycle and the ethylmalonyl-CoA pathway and therefore had to use another metabolic pathway to produce amino acids from acetate. The researchers used proteomics methods to find out which proteins were produced in larger quantities in H. marismortui when grown in acetate. As a control, the researchers used cells grown in a medium containing succinate, a four-carbon molecule that can be used by the microbes to directly synthesise amino acids. Berg and his team discovered some proteins that were upregulated under conditions where acetate is available as the only source of food. The researchers therefore concluded that these enzymes were involved in converting C2 compounds into C4 compounds. Since the enzymes' function had previously been unknown, the researchers further assumed that the enzymes had to be part of a new chemical reaction chain that enabled the conversion of acetate.

Creative gathering of ideas

The comparison of H. marismortui gene sequences with those of other organisms revealed similarities with already known enzymes. Although the functions of the newly discovered proteins had been unknown, they nevertheless had some relatives. And it was exactly this relationship that helped Berg and his team to decipher the role of the proteins in the new metabolic pathway. The researchers found a sequence of three reactions that led to the conversion of a glutamate molecule into mesaconyl-CoA, a form that can be further processed into succinate. This reaction chain can therefore be seen as kind of a module that is part of the entire metabolic cycle that leads to the conversion of two acetate molecules into glutamate and subsequently into succinate, i.e. entry points for the direct synthesis of amino acids. Berg and his team concluded that all steps involved in the conversion of acetate into glutamate, as well as all other parts of the microbial substance cycle had to be present in already known metabolic pathways. The H. marismortui metabolic cycle therefore combines reactions that originally belonged to different metabolic processes in different groups of prokaryotes and results in a completely new function.

But how did a reaction cycle consisting of other different metabolic pathways evolve? Berg and his team compared the genes of the enzymes in question with the genes of known organisms and found that different parts of its metabolic cycle are present in bacteria that are evolutionarily quite different from H. marismortui. "Haloarcula marismortui microbes gathered together the genes for their metabolic pathway from other microorganisms and came up with a metabolic pathway that enabled them to acclimatise to their salty habitat," said Berg. Microorganisms have the ability to take up free DNA from their environment. Therefore, it can be assumed that H. marismortui has, purely by chance, taken up the right genes and incorporated them into its genome (a phenomenon known as lateral gene transfer), thereby enabling it to produce amino acids from acetate. The chance



Methylaspartate cycle discovered by Dr. Ivan Berg and his group, starting from acetyl coenzyme A (CoA). The three known steps from glutamate to mesaconyl-CoA are within the red frame.

recombination of these genes in an ancestor of salt-tolerant archaea led to this new metabolic pathway and hence gave it the decisive advantage of being able to survive in the salty conditions of the Dead Sea. "The way evolution works can be compared to someone who tinkers with things and finds improvised solutions to pressing problems in any way he can; they can lay their hands on and fashion something that works," said Berg going on to mention the building-blocks game LEGO and that evolutionary scientists refer to this principle as "evolutionary tinkering", in other words, the idea that evolution is not a perfect engineer that plans everything from the outset, but rather someone who tinkers around with things.

Open to the possibility of entering new habitats

Four members of the Department of Microbiology have participated in Berg's projects to date. The researchers are interested in different aspects of bacterial metabolic pathways and also in new ways of CO2 fixation in organic material. In pursuit of the answers, they are investigating different microbial species from different habitats. The head of the Department of Microbiology, Prof. Dr. Georg Fuchs, will retire in spring 2011. Berg has not yet established his own research group, but would like to do so now. He would also like to stay in Freiburg, but is open to the possibility of entering new

"habitats".

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