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Bioeconomy BW

DFG research group to unravel prokaryotic immune system

It is difficult to believe that unicellular organisms such as archaea and bacteria can have developed sophisticated strategies to fight off foreign nucleic acids. However, many of these tiny organisms actually possess a virus defence mechanism known as CRISPR/Cas. Compared to this defence mechanism, protective mechanisms such as restriction and modification appear extremely clumsy indeed.

The CRISPR/Cas immune system used by bacteria and archaea to successfully fight off viruses was discovered as late as 2007 and it is yet to be fully understood and investigated in detail. A DFG (German Research Foundation)-funded group of researchers (FOR 1680: Unravelling the Porkaryotic Immune System) now hopes to change this situation. Detailed understanding of the prokaryotic immune system, which can in principle be regarded as "self vaccination", has the potential to be used in industry, in such areas as the protection of production strains in the dairy industry or for the production of fuel.

Sophisticated defence system

Prof. Dr. Anita Marchfelder, spokesperson of the Ulm group of researchers.
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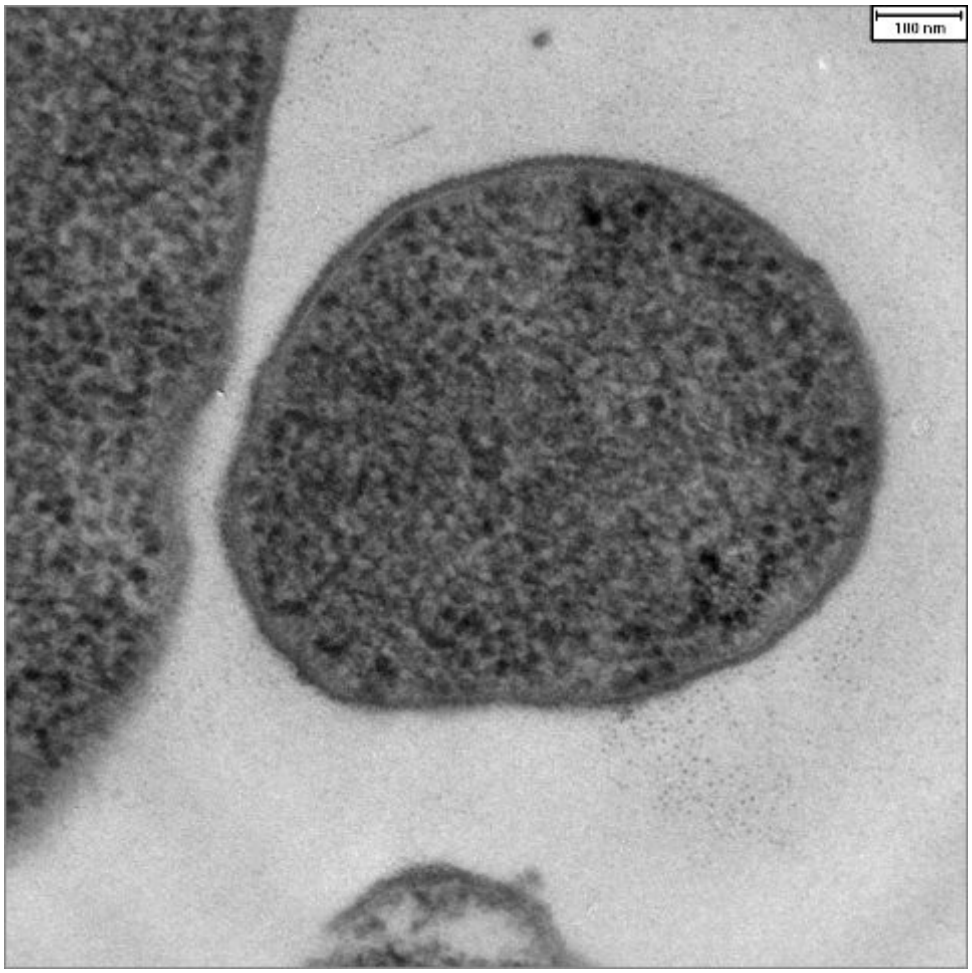
CRISPR stands for "clustered regularly interspaced short palindromic repeats" and describes short sequence repeats and stretches (known as spacers) that specifically recognise and destroy foreign genes. Cas proteins are often associated with CRISPR repeat-spacer arrays. Fifty per cent of all bacteria investigated possess this "vaccination system". It is not known why the other fifty per cent of prokaryotes do not possess CRISPR/Cas. Nine out of ten archaea have been found to use CRISPR/Cas against viral attacks. It is not impossible that in the course of their investigations the researchers will identify a hitherto unknown CRISPR/Cas system.

The basics need to be understood before applications can be considered



Marchfelder's model organism – the halophile bacteria Haloferax volcanii which exist in extreme saline environments
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The Ulm researchers are trying to unravel the CRISPR/Cas defence system using six different organisms, which were chosen with application potential and technical availability in mind. In the second phase of the project, which is receiving a total of 1.5 million euros in funding from the DFG over a period of six years, the research group plans to involve small companies in their activities in order to gather information on the system's industrial applicability. The six organisms are suitable for both in-vivo and in-vitro experiments, offering a flexibility which goes a long way towards helping to obtain reliable results.

Anita Marchfelder, a molecular biologist from Ulm University and the spokesperson of the new research group, explains that the prokaryotic defence strategies can be grouped into three subtypes based on the molecular mechanisms used. The Ulm researchers are focusing on all three types. Some bacteria, including the archaeon Haloferax volcanii, possess one system, cyanobacteria two and Sulfolobus solfataricus as many as three. FOR 1680 consists of three subgroups: one group is working on bacteria (E. coli, cyanobacteria; Neisseria meningitides), another on archaea (Methanosarcina mazei; Haloferax volcanii, Methanococcus maripaludis; Sulfolobus solfataricus) and the third group contributes expertise such as mass spectrometry, structural biology and bioinformatics to the project.

Still using models

The researchers have already managed to understand roughly how the prokaryotic immune defence, with its many known variants, works. They have found out that proteins and RNA are involved and that two of the 42 proteins that have been discovered are highly conserved in all organisms.

As the researchers are mainly focused on basic research they have decided to avoid creating hypotheses on the prokaryotic defence system. They are however focusing on aspects that have arisen from other hypotheses, including the ability of CRISPR/Cas variants to repair DNA and regulate genes in the prokaryotic organism. Researchers around the world have also been discussing a hypothesis according to which the prokaryotic CRISPR/Cas immune defence is similar to eukaryotic RNA interference. Further evidence for this similarity has since been provided by the discovery of a type of "dicer" in subtype 2, which is typical of RNA interference.

Prokaryotes somehow vaccinate themselves at a particular point in time

It is assumed that prokaryotes are able to survive the deadly attack of viruses because they recognise the virus, excise part of the viral DNA and integrate it into their genome. The group of researchers from Ulm are focusing on clarifying the principal mechanisms of microbial self vaccination. The sequencing of the prokaryote genome with the CRISPR/Cas immune system will generate information about attacks it has survived in the past. It can be assumed that the greater the distance between a viral fragment and the most recently incorporated viral DNA fragment, the longer ago the attack and successful defence occurred. However, immunological memory has its limits; bacteria tend to secrete old foreign DNA at some stage.

The most interesting issues have not yet been solved

Little is yet known about the molecular mechanisms of the bacterial immune system. It is known that small RNA pieces need to be processed in order to direct the "destruction machinery" to the appropriate position. The genome of previous attackers is known: encoded by the bacterial genome, the viral information is transcribed into a long stretch of RNA before being cleaved into small fragments. Each of the small RNA fragments contains only the sequence of a specific attacker, thereby specifically binding the foreign DNA and exposing it to degradation by the Cas protein complex.

Marchfelder's model organism, the halophile archaeon Haloferax volcanii, has a defence system consisting of eight proteins and three gene loci for foreign RNA fragments, which also "relates" the story of 30 successfully opposed viral attacks.

Industry is investigating whether bacteria can be vaccinated

Although the work of the Ulm researchers is largely focused on basic research, it nevertheless seems to have major potential for application in industry. It comes as no surprise that companies such as Danish Biotech Danisco, now part of the US giant Dupont, are working to find out whether the bacterium Streptococcus thermophilus, which is used in the production of milk, cheese and other dairy products, can be vaccinated and hence made resistant to well-known viruses. This would potentially then prevent entire bacterial cultures and dairy production batches from being destroyed.

The "vaccination" of industrially relevant bacterial cultures also has the advantage that these phage-resistant microorganisms are not regarded as genetically modified. Based on findings of the Robert Koch Institute, the German Federal Office of Consumer Protection and Food Safety declared many years ago that bacterial "vaccinations" refer to the natural ability of bacteria to transfer DNA via conjugation (Federal Office of Consumer Protection and Food Safety, 2.7.2002, ref. no. 6790-05-01-60) and do not therefore fall under "genetic modification".

Marchfelder research group (from the left, clockwise): Elli Bruckbauer, Helena Frey, Dr. Susan Fischer, Ruth Heyer, Dr. Angelika Jellen-Ritter, Jutta Brendel, Britta Stoll, Kathrin Weiss and Lisa-Katharina Maier (centre).

Further projects to start in 2012

Further projects will be carried out by the group of researchers, starting in early 2012, at the Max Planck Institute (MPI) of Biochemistry (Prof. Dr. Elena Conti, Martinsried), the MPI of Terrestrial

Microbiology (Dr. Lennart Randau, Marburg) and the MPI of Biophysical Chemistry (Prof. Dr. Henning Urlaub, Göttingen), the Universities of Kiel (Prof. Dr. Ruth Schmitz-Streit), Freiburg (Prof. Dr. Wolfgang Hess, Prof. Dr. Rolf Backofen) and Würzburg (Prof. Dr. Jörg Vogel and Dr. Nadja Heidrich). The projects are also linked with a national (Dr. Ümit Pul, Düsseldorf) and an international project (Prof. Dr. Roger Garrett, Copenhagen, specialist in archaea and their viruses).

Literature:

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