CRISPR-Cas has more surprises in store

Since 2012, a DFG-funded research group called FOR1680 has been studying CRISPR-Cas, an immune system that unicellular bacteria and arachaea use to protect themselves against attacks from viruses and plasmids. Prof. Dr. Anita Marchfelder, a molecular biologist at Ulm University and coordinator of the FOR1680 research group, and many other researchers were surprised to find that prokaryotes incorporate the genetic material of enemies as a kind of self-vaccination and even pass this protection on to their progeny. It appears that the bacterial defence system can do even more. "Over the past few years, the CRISPR-Cas system has been shown to have several other functions." Another DFG-funded priority research project is expected to be set up in the not-too-distant future to look into these functions.

The CRISPR-Cas molecular mechanism was described for the first time in 2007 and has since inspired the research of Prof. Marchfelder and her colleagues at the University of Ulm. Within just a few years, CRISPR-Cas progressed through the basic research stage at unprecedented speed and, due to its ability to manipulate eukaryotic genomes, has become a standard in genetic research laboratories. The scientific journal Science even characterised 2013 as the year of the "CRISPR Craze" due to the sudden increase in publications and applications of this particular system, and in 2015 the same journal referred to the CRISPR-Cas system as the "breakthrough of the year". The ability of unicellular organisms to detect an invader when they first encounter one, then degrade it and subsequently integrate a piece of the invader's DNA into the CRISPR locus, making themselves "immune" to repeated attacks, is an amazing discovery. However, of even greater importance is the CRISPR partner Cas9's ability, along with single guide RNA (sgRNA), to detect and cut

Prof. Dr. Anita Marchfelder, spokesperson and coordinator of the DFG research group FOR1680.
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specific DNA sequences. It is a precise and rapid genome editing tool that can be used for a plethora of practical applications from industrial biotechnology applications to plant and animal breeding and treating genetic diseases.

Since 2012, the FOR1680 research group has made considerable contributions to our understanding of the CRISPR-Cas genome editing mechanism. The group has compared CRISPR variants of different bacteria and archaea with each other and the more defence systems they investigated, the more natural variants they discovered. The project has just been through a successful intermediate assessment and will continue to be funded until the end of 2017.

A long way to go before the system is understood in detail

The researchers use molecular, genetic and biochemical methods for their investigations. The seven subgroups in the main research group are focusing on microbiological aspects, bioinformatics, structural biology and mass spectrometry and work closely with four associated groups. Due to the publicity and hype surrounding CRISPR-Cas, it is easy to forget that rapid progress also has pitfalls. "No one has had time to really look closely at the basic mechanisms of the system," said Bo Huang, a biophysicist at the University of California in San Francisco. "As long as everything works, nobody is really interested in how and why."[1]

However, the FOR1680 project is aiming to unravel the prokaryotic immune system in bacteria and archaea. In order to optimise the genome editing tool the researchers need to know how an invader-specific crRNA, one of the most important components in CRISPR-Cas9 genome editing, recognises and interacts with the invader and how the invading nucleic acid is degraded by the Cas proteins. All this needs to be known in order to be able to prevent CRISPR-Cas from interacting with and cutting the DNA at unwanted sites (off-target).

The CRISPR-Cas system of unicellular organisms has some highly conserved features, but is also highly variable, especially as far as CRPSPR spacer, repeat sequences and lengths are concerned. The Cas proteins, of which Cas9 is the best known, show a remarkable degree of diversity. 93 Cas protein families are known to date, but little is yet known about their function and structure.

New classification of the 4000 known CRISPR-Cas loci

Subtypes like E. coli I-E have been studied in great detail while little is yet known about other subtypes now being looked into by FOR1680. In 2015, the FOR1680 researchers along with bioinformaticians in Rolf Backofen's group from Freiburg and researchers from abroad, classified more than 4000 known bacterial and archaeal CRISPR defence systems. They divided CRISPR-Cas systems into two classes, class 1 with three types and class two with two types. The five types are divided into 16 subtypes. However, the latest results suggest that it would be better to divide CRISPR-Cas systems into two classes, with six types and 19 subtypes.[2] These results also show that CRISPR-Cas systems have a modular design and evolve dynamically. 359 Cas proteins belong to protein families with sequences so different from each other that they are highly unlikely to have similar functions.

Genome editing attracted the interest of Marchfelder's research group when Cas9 pioneer Prof. Dr. Emmanuelle Charpentier joined the FOR1680 project. Charpentier discovered in 2015 that the

Cpf1 protein, nowadays seen as an effective alternative to Cas9 because it is shorter and only requires one instead of two RNA molecules, also plays a role in CRISPR.

CRISPR-Cas is very differently distributed

Meanwhile, the FOR1680 researchers have acquired further fundamental knowledge about CRISPR-Cas systems. The researchers' analyses revealed that CRISPR-Cas is present in half of all bacteria and in almost all archaea (9 out of 10). There has been heated debate in the research community concerning the reasons for this difference. From an evolutionary perspective, taking up foreign DNA is especially advantageous for pathogenic organisms. However, if a bacterium continues to incorporate foreign DNA in its DNA, it gradually becomes larger, and this might have an effect on its "fitness".

Autoimmune disorder in bacteria?

The research group has come up with surprising findings that are now the subject of lively debate. Bioinformatic analyses have led to an odd result: part of the inserted foreign DNA can also be directed against the bacteria's own genome. While this usually leads to the death of bacteria, the incorporation of foreign DNA in archaea triggers the activity of repair systems. "The invader DNA can cause bacteria to die relatively quickly," explains archaea specialist Anita Marchfelder. The CRISPR-Cas system could well be associated with this outcome. In archaea, however, the incorporation of genetic material not only does no damage, but may also be a step towards gene regulation. In this field of research, bioinformatic approaches are gaining in importance for investigating all inserted invader sequences; this may well lead to the discovery of previously unknown viral genomes. But there's more, as Marchfelder explains: halophilic archaea live in salty habitats and are able to fight one another using the integrated pieces of foreign DNA. Sometimes they fuse, exchange DNA and possess spacers, i.e. DNA fragments from viruses or plasmids, that are directed against other archaea. At the current stage of research, it is not yet possible to know why this happens.

Protein complex probably has other functions in unicellular organisms

CRISPR-Cas comes in many variants. Archaea do not have CRISPR-Cas9, which is currently the best-studied of all CRISPR-Cas systems. This suggests that Cas9 has something to do with the bacteria's virulence, as Prof. Marchfelder explains. Some bacteria even have different versions of CRISPR-Cas. The presence of three equally effective defence systems in one and the same organism has led to the thesis that CRISPR-Cas must also have other functions.

Due to its diversity, it is impossible to establish a common molecular CRISPR-Cas mechanism. The system cleaves DNA in nine out of ten cases. However, the process can be completely different, as can the proteins involved. Cas1 is the only protein (seen as essential for the bacteria's to adapt to new attackers) that was found in all organisms investigated. The other Cas proteins were quite different in each case. Only a handful of nucleases such as Cas9 are known. Marchfelder would not be surprised if the researchers made other discoveries

"In recent years, evidence has accumulated that suggests that the CRISPR-Cas system has other functions as well," says Marchfelder. In pathogenic organisms, the Cas9 protein plays a role in the regulation of genes involved in virulence. CRISP-Cas system is also involved in the regulation of biofilm formation and in the repair of DNA. Some organisms possess only parts of the protein

complex, e.g. naked CRISPR RNA. The new DFG project will also focus on the defence mechanisms that are directed against the prokaryotes themselves and provide further insights into the many functions of the CRISPR-Cas system.

[1] Heidi Ledford, Gentechnik: CRISPR verändert alles, in: Spektrum der Wissenschaft, 24.06.2015

[2] Mohanraju, P.; Makarova, K. S. et al.: Diverse evolutionary roots and mechanistic variations of the CRISPR-Cas systems, Science, 05.08.2016, DOI: 10.1126/science.aad5147

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Further information

- DFG-funded project FOR1680

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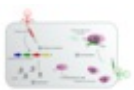

CRISPR/Cas – genome editing is becoming increasingly popular

bioinformatics | virus | bacterium | RNA | CRISPR/Cas