Scientists discover function of Cas4 protein in CRISPR-Cas defence systems

March 29 2018

Credit: Getty Images/TU Delft

Researchers around the globe have increasingly been using a bacterial defence mechanism called CRISPR-Cas9 as a tool to surgically edit DNA in living cells. This new technique has made gene editing a lot easier and more precise. But how these systems function in nature is still not fully understood. Researchers at Delft University of Technology have now determined the role of one of the proteins involved in many CRISPR systems, namely Cas4. As it turns out, this protein helps form memories of invading viral elements so that the bacterial cell is protected from virus infection. Virus memories obtained with Cas4 help the cell to find and destroy the invading virus fast enough to survive. The discovery is another important step towards a complete understanding of CRISPR systems.

It is impossible for a human eye to see, but viruses and bacteria are at war with each other. Every day, tiny virus-like particles called bacteriophages kill about one third of the bacteria in the earth's oceans. "Such viruses inject their DNA or RNA into a bacterial cell to try and take control of it," explains Dr. Stan Brouns of Delft University of Technology. "If this hijacking is successful, the virus is able to use the bacterium as a small factory that produces copies of itself."

Genetic memory

As a counter to viruses, evolution has equipped bacteria with defence mechanisms such as CRISPR-Cas9, which have the ability to cut viral DNA, thus neutralizing the threat. Before this can happen, though, a bacterium needs to have a genetic memory of a virus. It has to recognize the virus as a threat.

Such memories are formed as a bacterium seizes snippets of the virus' DNA and inserts them into its own genetic code. If the same type of virus attacks the bacterium again, the cell recognizes the invader and dispatches a cutting protein such as Cas9. Using RNA-copies of the viral snippets as a kind of "cheat sheet," the protein starts hunting – and destroying—the virus, keeping the bacterium safe.

Virus DNA snippets

Scientists already knew that several proteins are involved in every CRISPR system. Until now, however, the role of the protein known as Cas4 has been unclear. "We knew it had to be important, though, since this protein is present in the majority of CRISPR systems," says Ph.D. candidate Sebastian Kieper, who led the project with Cristóbal Almendros.

In order to find out what Cas4 actually does, the scientists introduced the Cas4 gene in E. coli cells containing the main CRISPR memory-forming machinery. In other cells, they did not introduce this gene. Brouns: "We found out that the bacterium still formed memories of an invader in the absence of Cas4, but that those memories were not providing protection." In other words, only when Cas4 was present, snippets of invading DNA were inserted in the genome of the bacterium that actually conferred virus resistance to the bacterial cell.

Functional memories

Further studies revealed the reason why bacteria cannot use the memories they form without the use of Cas4. This had to do with PAMs (Protospacer Adjacent Motifs), short DNA sequences consisting of a small number of base pairs that act as a point of recognition for proteins such as Cas9. "Anyone doing genome editing with Cas9 will know that selecting a PAM is crucial for the success of the experiment," says Brouns.

Bacteria face the same problem. "When selecting a piece of DNA to insert into the bacterial genome as a memory, a CRISPR system needs to pick a sequence that is flanked by a PAM as well," explains Brouns. "A Cas protein will use the PAM sequence to find its target. It will not target DNA sequences that are not flanked by a PAM." Thus, PAMs also act as safeguard, preventing Cas proteins from cutting a bacterium's own DNA.

The role of Cas4 is to make sure that only sequences that are flanked by a PAM are inserted into a bacterium's DNA. This leads to functional memories that can actually be used when the virus once again tries to attack the bacterium.

The researchers are now looking into how this process works, and how this finding can be exploited. Perhaps adaptive immunity can be introduced in organisms other than microbes that do not naturally possess a CRISPR defence system.

More information: Sebastian N. Kieper et al. Cas4 Facilitates PAM-Compatible Spacer Selection during CRISPR Adaptation, *Cell Reports* (2018). DOI: 10.1016/j.celrep.2018.02.103

Provided by Delft University of Technology