CRISPR – The tool that will stop multi-resistant bacteria once and for all?

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Some say it's the new antibiotics, others claim it is an invaluable tool in the effort to stop the seemingly inevitable spread of multi-resistant strains of pathogenic bacteria such as MRSA (Methicillin-resistant *Staphylococcus aureus*). Some use it for making yoghurt. CRISPR is an abbreviation for Clustered Regularly Interspaced Short Palindromic Repeats. It is a relatively new research field, focusing on the recently discovered adaptive immune system in prokaryotes.

CRISPR- What is it?

CRISPR is an RNA based immune system found in almost half of all sequenced bacteria and more than 90% of all sequenced archaea. It is adaptive, just like our own immune system, although it is also fundamentally different. Just like any other organism, bacteria and archaea are under constant threat of viral infection. It's almost hard to believe, but there are actually several times as many viruses as there are bacteria.

A phage will infect a prokaryote by injecting some of its own DNA or RNA into the cell, with the goal of either destroying or capturing the cell. A captured cell will be used against its own will, forced to produce more virus.

How does it work?

Just as we eukaryotes, prokaryotes have through millions of years of thorough selection and evolution developed a complex immune system. One of the major lines of defense in the prokaryote immune system is CRISPR. By degrading the invading nucleic acid into smaller pieces, the CRISPR system can integrate them into what is called the CRISPR locus (see figure 1). This is the segment that functions as a memory bank for hostile DNA or RNA the cell has encountered. The specific sequences are stored as spacers in a unique repetitive pattern, flanked on both sides by repeats. This pattern is repeated each time a new invading sequence is added. At the proximal end of the CRISPR locus, another functional domain is

found. The leader operon is thought to be used as a promoter, both for the process of adding new repeat-spacer segments, as well as for the eventual transcription.

Once attacked, the CRISPR system will immediately activate. The locus is transcribed into tiny CRISPR RNA (crRNA) – each one matching the specific sequence of a previously encountered invader.

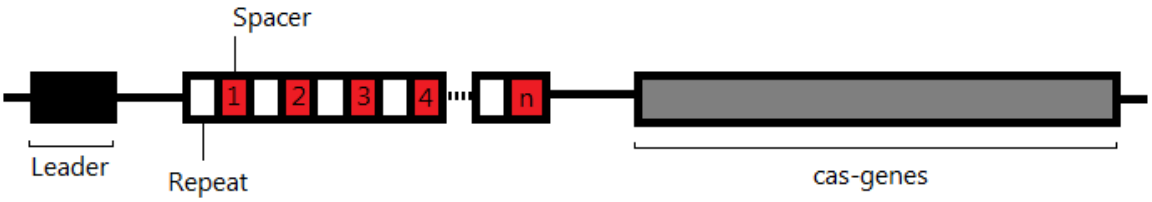

Figure 1. Schematic representation of the principle and components of the CRISPR locus. The repeat-spacer segment stores all the information from previously encountered nucleic acids. The leader operon functions as a promoter, while the cas-proteins are the workhorses of the whole system.

cas-proteins

Opposite to the leader side of the CRISPR locus is a cluster of extraordinary genes. They translate into a very special group of proteins – cas-proteins (CRISPR associated proteins). The cas-proteins are basically the workhorses of the CRISPR system. They help to facilitate each of the steps involved in defending the cell, and are integral to its success. There are up to 25 different cas-proteins, although each CRISPR-system generally only has a few select genes, depending on which organism it belongs to.

Some of the cas-proteins converge to form a large complex called Cascade (CRISPR-associated complex for antiviral defense). Cascade utilizes the crRNA previously transcribed from the CRISPR locus to recognize and eliminate foreign nucleic acids, essentially playing the part of an antibody or T-cell.

The history of CRISPR

CRISPR was first discovered in *Escherichia coli* in 1987. A group of scientists from Japan happened to stumble upon it by mere coincidence. It was recognized as a group of repeating, yet seemingly unrelated sequences.

Although several studies were made, therein the discovery of the cas-genes in 1999, it was not until near two decades later, in 2005, that the purpose of the CRISPR locus was divulged. Two years later, a study funded by Danisco provided the first experimental proof of how the CRISPR-system provides phage resistance.

Using CRISPR as a tool...

As the threat of multi-resistant pathogenic bacteria grows larger every day, there is a race against time to find a sustainable solution. Most resistance genes are spread through Horizontal Gene Transfer (HGT). HGT includes genetic transfer through conjugation, transformation and transduction. HGT is the cause of most of the multi-resistant strains of bacteria running

rampant as of now. It has been theorized that CRISPR could be used as a powerful tool to stop the spread of resistance genes, if used as a compliment to antibiotics.

…for dairy?

In addition to fighting multi-resistant pathogens, there are a multitude of other uses for the CRISPR-system. One of these is securing the production of food products. A lot of food is produced to some extent using bacteria, either for fermentation or acidification. This means for example, that the production of dairy is highly susceptible to phage infection. Infection of a starter culture could mean large delays and reduced quality of the product. For this reason, large dairy companies such as Danisco is actually one of the largest investors in the field of phage resistance and prokaryote immune systems.

…for microbiology?

CRISPR is currently being used as a tool for strain classification, using a method called Spoligotyping (Spacer oligonucleotide typing), and it is also theorized that the CRISPR system could be of large importance to the field of microbiology as a whole, having potential to be a tool of almost surgical precision for gene suppression.

Further reading

Nordling L. 2013. CRISPR/cas-systemet – ett vaccinationskort för prokaryoter? Självständigt arbete i Biologi 2013. Uppsala Universitet.