Transcript - Innovirology session 8.1 Basics of the CRISPR system

Slide 1:

CRISPR is a microbial anti-viral defence system. The Cas9 CRISPR protein has been widely applied as a genome editing tool. In this session the basics of CRISPR as an anti-virus mechanism are explained. It is shown how CRISPR can adjust to new viral infections and adequately combat viruses. In the last part of this session we discuss how autoimmunity can be avoided

Slide 2:

Viruses are the most abundant entities on this planet and outnumber their host at least by a factor 10. Consequently, in their natural environment microbes are under constant danger of viral infection. To defend themselves against the possible negative impact of viral infection, bacteria and archaea, like humans, have evolved a range of different defence strategies. This slide summarizes the known viral defence strategies: 1) adsorption block, 2) abortive infection, 3) uptake block, 4) Restriction Modification systems and 5) the CRISRP system.
The CRISPR system is present in about half of all bacteria and most of the archaea. There exist many different CRISPR types of which the Cas9 based system is the most famous. The basic mechanism of defence is largely similar for all families. The differences rely on the different CRISPR-associated (Cas)-genes used to classify the several families. Some systems target foreign DNA others RNA.

Slide 3:

The discovery of CRISPR started with the first description of Clustered Regularly Interspaced Palindromic Repeats in the genomes of bacteria and archaea. For long, these repeats were seen as uninteresting genomic regions, until in 2005 several groups simultaneously reported that the ~30 bp spacers in between these palindromic repeats, contained sequences that were matching exactly to those of viral genomes. At that moment it was proposed that this CRISPR arrays might be part of an antiviral defence system. This slide depicts a typical CRISPR array. CRISPR arrays can contain between 2 to several hundreds of repeats. Depending on the CRISPR family the size of the spacers and repeats varies a little. Several different CRISPR array can be present in a single bacterial or archaeal genome. Each spacer is unique and matches to an individual virus or extrachromosomal element. The Cas genes are often encoded in close proximity of the CRISPR arrays.

Slide 4:

The first proof that CRISPR indeed functions as an anti-viral defence system came from research on phage-resistant bacterial strains in de dairy industry. Researchers showed that the only difference between a virus-susceptible and virus-resistant strain was the presence of one additional spacer in the CRISPR array of the resistant bacteria, shown in red on this slide. This spacer matched 100% to a region in the viral genome to which the strain had gained resistance. This region is called ‘protospacer’. The role of the PAM will be explained on the last slide.

The addition of new spacers matching a certain virus, will render the strain resistant in future. As these adaptations in the CRISPR system are made in the genome, this resistance will also be inherited by offspring of the strain. As such the CRISPR system functions as an adaptive and an inheritable defence system.

Slide 5:
On this slide the first phase of the CRISPR defence mechanism is demonstrated with the example of Cas9. The first stage of CRISPR mediated defence is defined as the 'adaption' phase. After infection with a virus, the foreign DNA is recognized and cleaved by Cas proteins. A small portion of the genome is incorporated as a new spacer in the CRISPR array. New spacers are generally added in the CRISPR array, near the leader sequence, which is shown in grey on this slide. As such the CRISPR array gives an overview of past viral encounters of a strain. Spacers are occasionally removed from the CRISPR array by recombination events that can occur in the middle or towards the end of the array.

Slide 6:
In the second phase of the CRISPR defence mechanism the CRISPR array is transcribed in a long RNA molecule. Cas9 can process the pre-CRISPR RNA. In other types of CRISPR families, this RNA processing occurs by different Cas proteins. Cleavage of the pre-CRISPR RNA occurs with the help of a trans-activating CRISPR-RNA (tracrRNA). This tracrRNA hybridizes specifically with the repeats in the pre-CRISPR RNA that is bound by Cas9. This events recruits a host encoded RNAse that eventually processes the pre-crRNA in small guide sequences. In other types of CRISPR system this cleavage can also occur by a Cas protein. The guide RNA sequence remain bound to the Cas protein.

In the third phase of the CRISPR defence mechanism the virus genome is targeted and eliminated. The Cas9-guide RNA complex scans cellular DNA using its crRNA containing a single spacer as a guide. As soon as it finds a perfect match the DNA is being cleaved and as such the viral infection is halted. The ~30 bp sequences provide enough specificity to not target the bacterial or archaeal own genome. However, in theory there is one position with a perfect crRNA match in the bacterial genome. This is the CRISPR array itself. Targeting of this region could lead to auto-immunity. To avoid auto-immunity the PAM sequence is important.

Slide 7:
On this slide the mechanism of PAM is explained. PAM stands for protospacer adjacent motive. Usually it consist of only a few base pairs. It is located adjacent to the protospacer in the viral genome (shown in red). As a rule it is not present adjacent to the spacers in the CRISPR array. DNA cleavage by the crRNA loaded Cas complex only occurs when a PAM is present. Therefore the PAM represents an effective mechanism to avoid auto-immunity.