8.2 CRISPR-Cas: From biology to tool

Slide 1.

CRISPR-Cas is seen as one of the biggest game changer in molecular biology since PCR. In this presentation, I will show you how this bacterial adaptive immune system formed the basis for the development of a new powerful molecular tool for editing, regulating and targeting genomes. Today, the CRISPR technology has got its applications in all fields of genome engineering. The success of the technology is due to its simplicity, efficiency and versatility.

Slide 2.

The CRISPR technology is based on RNA-guided engineered nucleases cleaving the DNA in a sequence-dependent manner. The mode of action consists of three distinct steps. In the first step, the acquisition stage, foreign nucleic acids are integrated as new spacers into the CRISPR array that is separated by repeat sequences. In the next step, the expression stage, mature crRNAs are generated that contains partial spacer sequences joined to partial repeats. In addition, tracrRNA that has complementarity to the repeat regions of crRNA transcripts, as well as Cas proteins are expressed. In the interference stage, a crRNA-tracrRNA hybrid is formed through binding of the complementary repeat region. This RNA hybrid guides the Cas nuclease towards the complementary DNA sequence, which leads to the cleavage of the invading genetic element.

Slide 3.

It was the combination of the crRNA and tracRNA into one sgRNA that formed the crucial step in the development of the CRISPR technology. The most widely used system in genome engineering is the type II CRISPR/Cas system from *Streptococcus pyogenes*. Like other class 2 systems, it only needs a single endonuclease, Cas9, to cleave the targeted DNA.

Slide 4.

CRISPR-Cas9 is nowadays used to modify the genomes of multiple cells and organisms, including bacteria, parasites, zebrafish, mice and human cells. Co-expression of the Cas9 nuclease with sgRNAs containing the complementary sequence, results in double-strand DNA breaks that are subsequently repaired by either non-homologous end joining or homologous recombination.

With non-homologous end joining random base pairs are inserted or deleted, causing gene knockout by disruption. On the other hand, homologous recombination with a donor DNA can be used to modify a gene by introducing precise mutations or introducing a whole new gene in the genome.

Slide 5.

The ability to program Cas9 to bind any desired sequence can be exploited for various other applications, such as controlling transcription, modifying epigenomes and imaging chromosomes.

For imaging in live cells, a modified Cas9 protein, called dCas9, was constructed. In this protein the nuclease domains are deactivated, resulting in a sequence-specific DNA binding protein. The modified protein is fused to a fluorescent tag, like eGFP, and together with an engineered sgRNA, it is used to visualize the genome in single cells. This capability improves the current technologies for studying the conformational dynamics of native chromosomes in living cells.

On the other hand, the modified protein dCas9 can also be used for gene regulation on a genome-wide scale. In a process known as CRISPRi, the RNA polymerase access to DNA is blocked by dCas9, resulting in transcription repression. Furthermore, by generating chimeric versions of dCas9 that are fused to regulatory domains, such as the ω-subunit of the RNA polymerase, gene expression can be efficiently activated.

Slides 6.

The discovery and development of the CRISPR technology is the work of many scientists from around the world. Although the technology is a relative recent discovery, the first reports go back to 1987. Scientists at the university in Japan discovered an unusual segment of neighbouring DNA that consisted of short, direct repeats flanked by short unique sequences. They noted that 'the biological significance of the sequences is not known'. Almost three decades later, in 2002, these repeats were termed CRISPR and their associated proteins were named Cas. In 2005, the CRISPR spacers were matched to foreign DNA.

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In the next two years, CRISPR was found to be the adaptive immune system of bacteria used to avoid phage infection. In 2011, scientists discovered that for this phage resistance mechanism two RNA molecules, called crRNA and tracrRNA, are required. By 2012, biochemical characterizations of the CRISPR system showed that Cas9 can be guided by these crRNAS to cleave target DNA *in vitro*. Moreover, crRNAs were fused to tracrRNAs to form sgRNAs, reducing the system from three to two components. From this point, CRISPR/Cas9 was used as new gene editing tool.

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In the following years, many studies showed the potential of this new technique for precise genome engineering. In 2013, the CRISPR/Cas9 system was used to edit targeted genes in both human and mouse cells using designed crRNAs. A year later, researchers in China reported the first monkeys engineered with targeted mutations, a big step forward in the making of more realistic research models of human diseases. Also, first attempts to edit the genome in human embryos were performed. However, the researchers were unable to effectively repair the genetic disorder and many off-target cleavages were observed.

Slide 9.

In 2016, the first human trial to use CRISPR gene editing gets approval in the United States. This first trial is small and only designed to test whether CRISPR is safe for use in people. Later, the technique might be used to treat cancer.

As the timeline shows the CRISPR technology is a groundbreaking technology in molecular biology, and many new studies and applications will still come in the next years.

Slide 10.

You can find all references used in this presentation in following list.