Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

DISCIPLINE: «Modern Problems of Plant Genetics»

Lecture 15

CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture.

Amirova Aigul
Associate professor
Candidate of Biological Science

Charpentier and Doudna

In 2011, biologists Jennifer Doudna and Emmanuel Charpentier decided to study the CRISPR mechanism more closely. They discovered that the *Cas9 protein* could be tricked by giving it artificial RNA.

A protein carrying such RNA will look for genetic fragments that match what it carries.

Having found a match with someone else's DNA, it will begin to grind it, regardless of who it belongs to - a virus, a plant or an animal.

In February 2013, it was proven that CRISPR/Cas9 can be used to edit DNA in mouse and human cell culture. Moreover. It turned out that the technology allows not only to remove unnecessary genes, but also to insert others in their place. To do this, it is enough to add enzymes that restore DNA.

CRISPR/Cas system

CRISPR (clustered regularly interspaced short palindromic repeats - short palindromic repeats regularly arranged in groups) are special loci in bacteria and archaea, consisting of direct repeating sequences that are separated by unique sequences (spacers).

General principles:

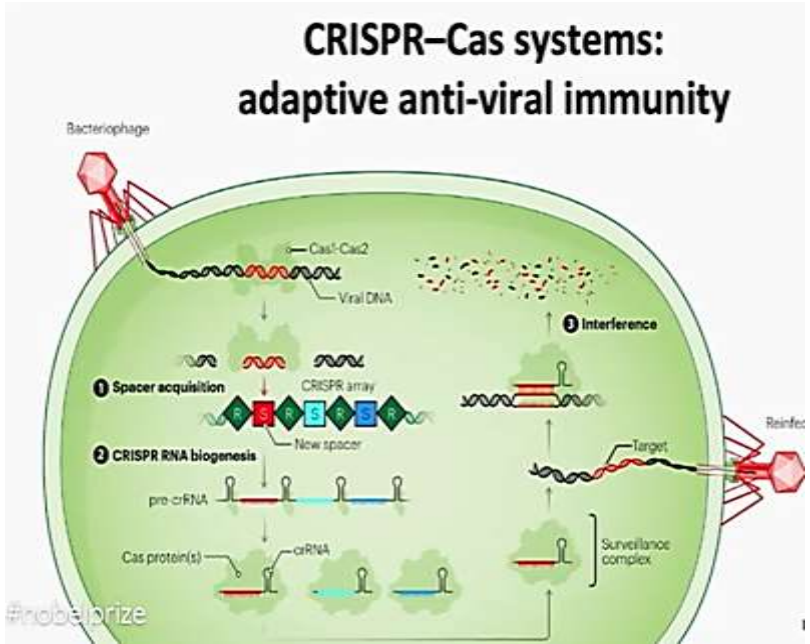
I. Structural similarity

II. Key stages of CRISPR-mediated immunity:

1. acquisition or adaptation

2. expression

3. interference



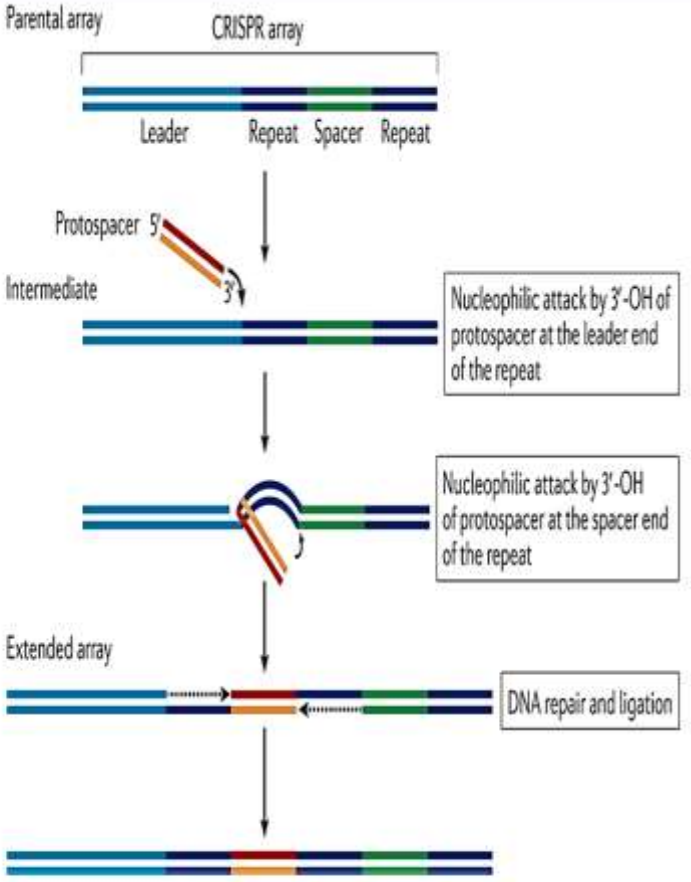
Acquisition

Spacers constitute an immunological memory that stores information about past infections and underlies the response to repeated invasion by similar genetic elements.
Most of the data on the molecular mechanisms of the acquisition of new spacers were obtained from studying the type I CRISPR of *Escherichia coli* and type II CRISPR systems of Streptococcus thermophilus.

❑ Correct orientation and insertion of a new spacer occurs with the participation of the sequence located immediately upstream of the first repeat. Thus, new spacers are added to the 5' end of the CRISPR locus.

❑ Integration of a new spacer into the space between the leader sequence and the first repeat is carried out by the Cas1-Cas2-protospacer complex.

❑ In some CRISPR-Cas systems, additional proteins are involved in this process. When a new spacer is inserted, the repeat is duplicated, thereby maintaining the correct structure of the locus, which should begin with a repeat.



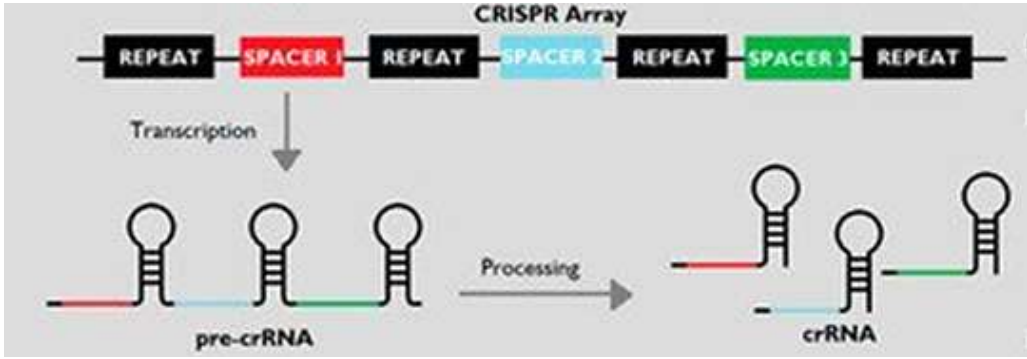
Expression and formation of crRNA

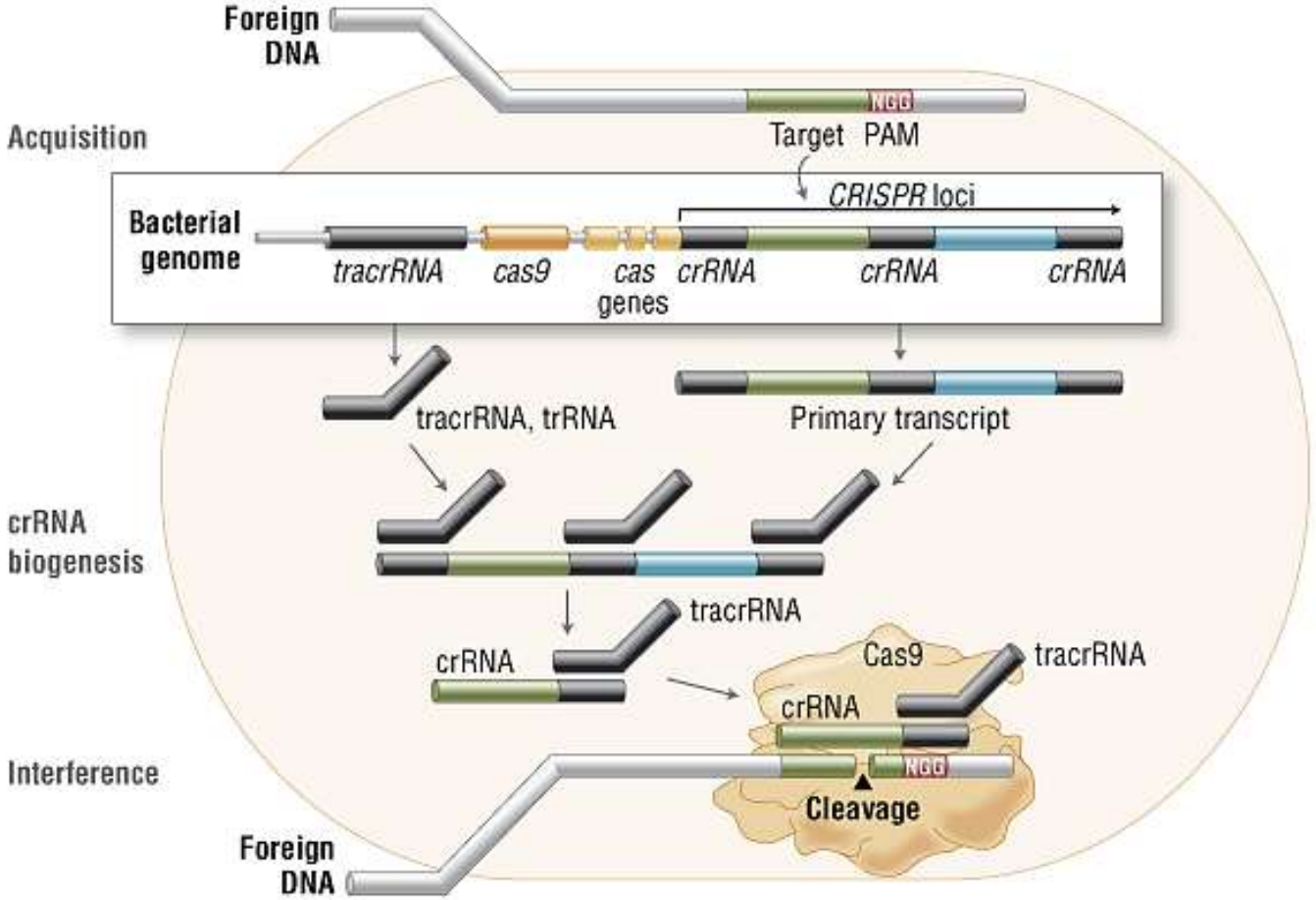
❑ A number of CRISPR repeats and spacers are transcribed into a single long transcript - pre-crRNA.

❑ Pre-crRNA is cut into short crRNAs.

❑ Most repeats in CRISPR are palindromic, so the corresponding sections of pre-crRNA form hairpins.

The main feature of the discovered tracrRNA is the presence of a 25-nucleotide region that is complementary to all CRISPR palindromic repeats.
This means that tracrRNAs bind to repeats, a process necessary for the formation of working crRNAs.





Advantages of CRISPR/Cas over other technologies

The two main alternative genome editing technologies, ZFN and TALEN, involve the use of chimeric nucleases consisting of DNA-binding and DNase domains.

To create ZFN and TALEN nucleases, a researcher must master a wide range of genetic engineering methods and have considerable practical experience.

In addition, each new editing project requires the development of at least two new enzymes.

In the case of CRISPR/Cas systems, to change the specificity of the nuclease, it is enough to simply synthesize another guide RNA.

APPLICATIONS FOR PRECISION PLANT BREEDING

Knockout-Mediated Crop Trait Improvement Eliminating negative elements is a promising strategy for genetic improvement. Therefore, knocking out genes that confer undesirable traits is the simplest and most common application of CRISPR/Cas9.

Traits that have been improved to date using CRISPR/Cas9 include yield, quality, and biotic- and abiotic-stress resistance.

Hybrid-breeding techniques and many other important aspects of crop productivity have also been enhanced using this approach.

Characteristics	ZFN	TALEN	CRISPR/Cas9
Component, responsible for recognition target area DNA	Protein domain	Protein domain	Guide RNA
Component, demanding design	Protein	Protein	Guide RNA
Specificity and efficiency	Low	Average	High
Non-target mutagenesis	Variable frequency	Low frequency	Average frequency
Price	High cost	Expensive	Low cost