Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

DISCIPLINE: «Modern Problems of Plant Genetics»

Lecture 10

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

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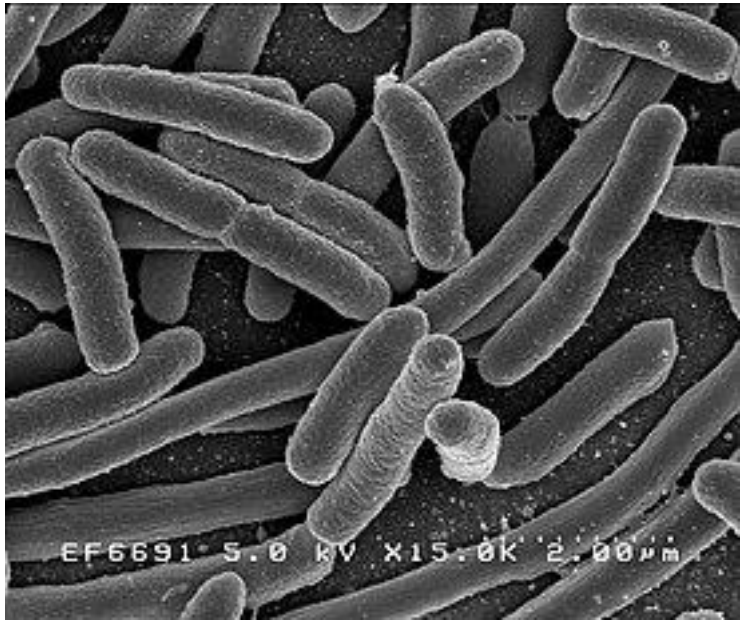
Aim of the lesson: **familiarization with CRISPR/Cas genome editing technique and its application.**

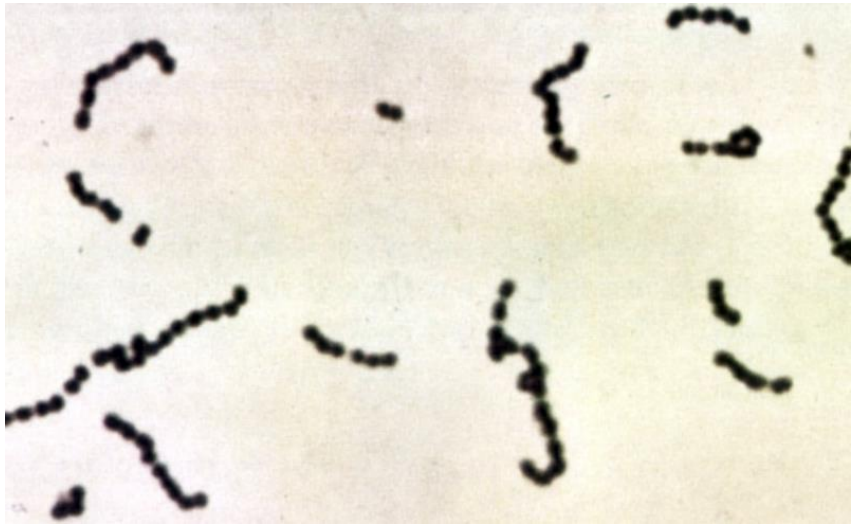
Plan of the lesson:

1. **History of discovery CRISPR/Cas genome editing tools.**
2. **CRISPR/Cas genome editing mechanism.**
3. **Applications for precision plant breeding and future prospects.**

History of discovery

- **The history of CRISPR began in 1987**, when Japanese scientists studying *Escherichia coli* discovered unusual repeating sequences in its DNA.
- Their biological significance could not be determined, but similar fragments were soon found in the genomes of other bacteria and archaea. The sequences are called **CRISPR** (Clustered Regular Interspaced Short Palindromic Repeats).
- Their function remained a mystery until 2007, when experts on the *Streptococcus bacterium*, which is used to make fermented milk products, determined that these fragments are part of the bacteria's immune system.





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.

В ДНК бактерий и архей выделяют особый участок - CRISPR-кассету. Она состоит из лидерного участка, регулярно повторяющихся (повторов) и уникальных участков ДНК (спейсеров). CRISPR-кассета вместе с cas-генами и кодируемыми ими cas-белками формирует CRISPR-систему [6]

Структура CRISPR-системы

Кодируют белки cas, необходимые для функционирования системы CRISPR/Cas

Отвечает за увеличение количества спейсеров после вирусных атак

Появляются после вирусных инфекций и необходимы для приобретенного иммунитета.

CRISPR/Cas genome editing mechanism.

If a virus penetrates a bacterium or archaea equipped with a CRISPR system, the adaptive functional module of the system is activated: specific *Cas proteins* - in all systems these are at least Cas1 and Cas2 - cut out the fragments they like from the stranger.

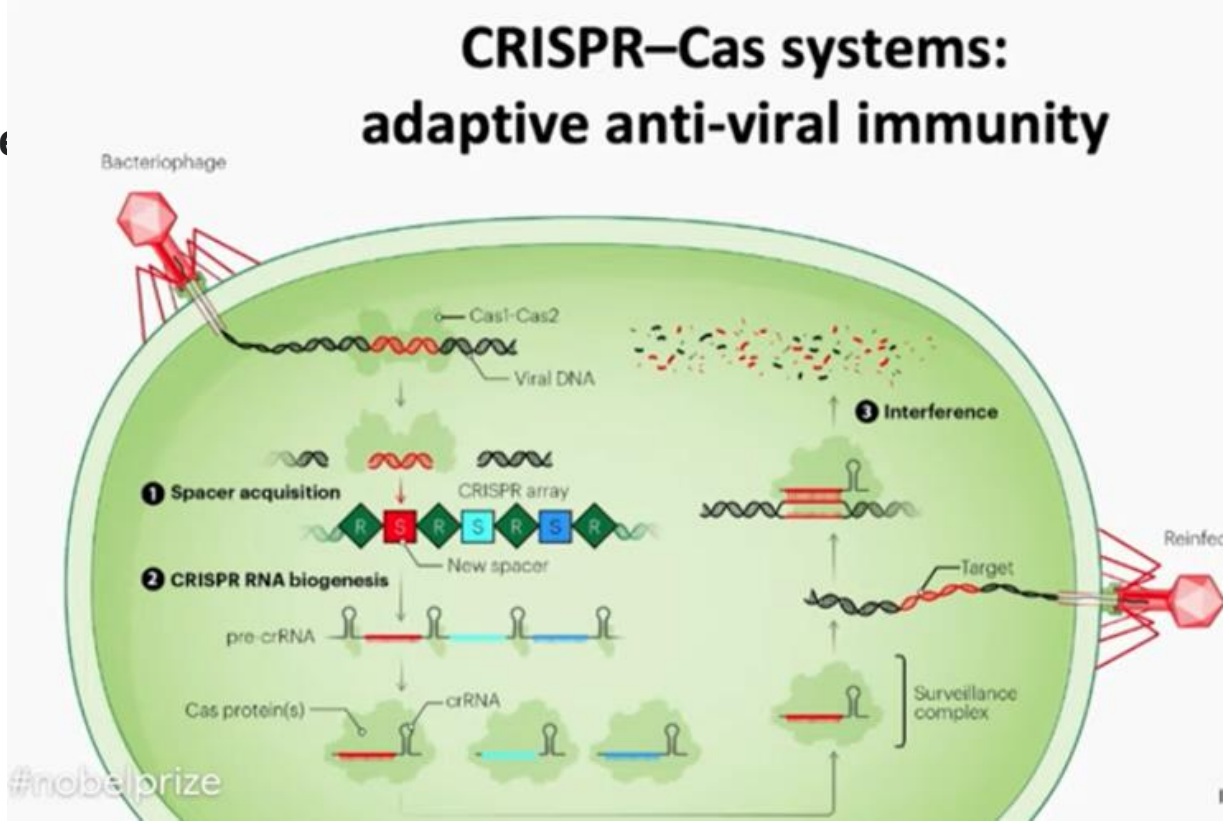
In some cases, an effector protein also helps to select a protospacer. Proteins select areas next to a special PAM sequence (protospacer adjacent motif) - just a few nucleotides, but different for different CRISPR systems.

Then these same adaptation proteins insert the fragment into the CRISPR cassette, always on one side - at the leader sequence.

This is how a new spacer is formed, and at the same time a new repeat. This whole process is called adaptation, or acquisition, but in essence it is remembering the enemy. Information about all remembered enemies is received during division by all the offspring of the cell.

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

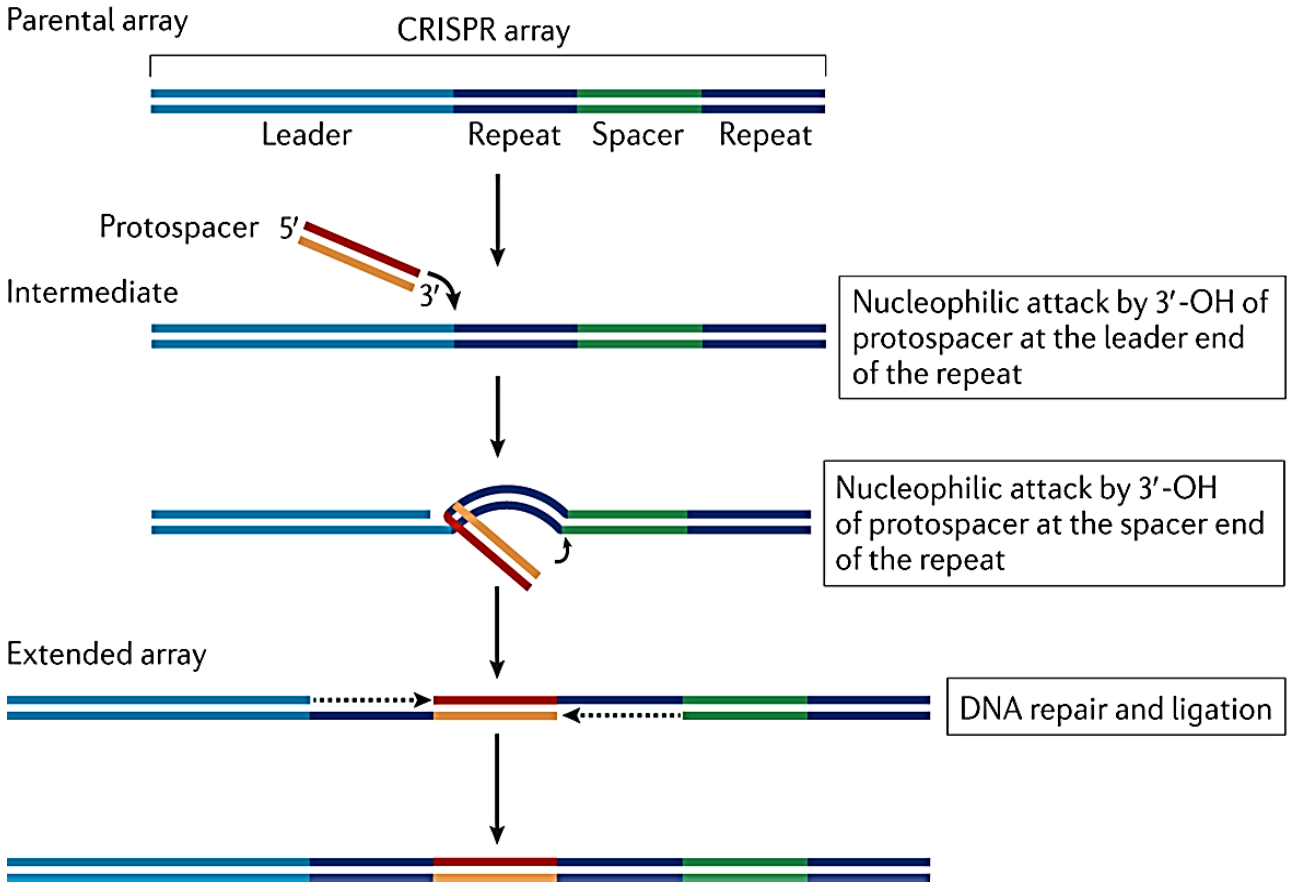
Acquisition

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.**



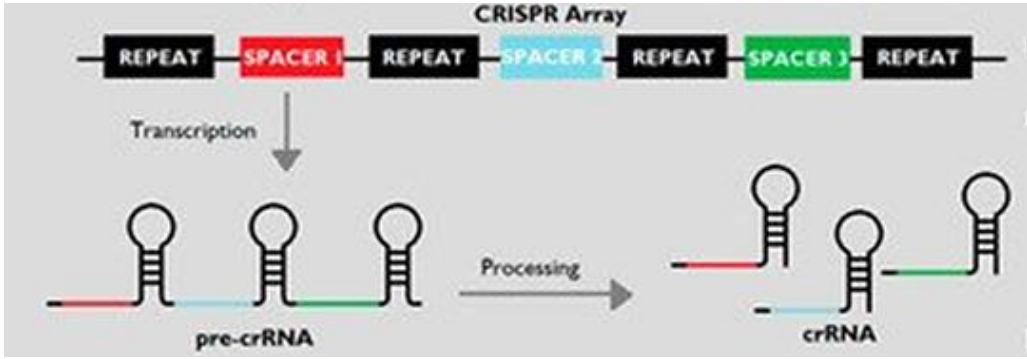
Expression and formation of crRNA

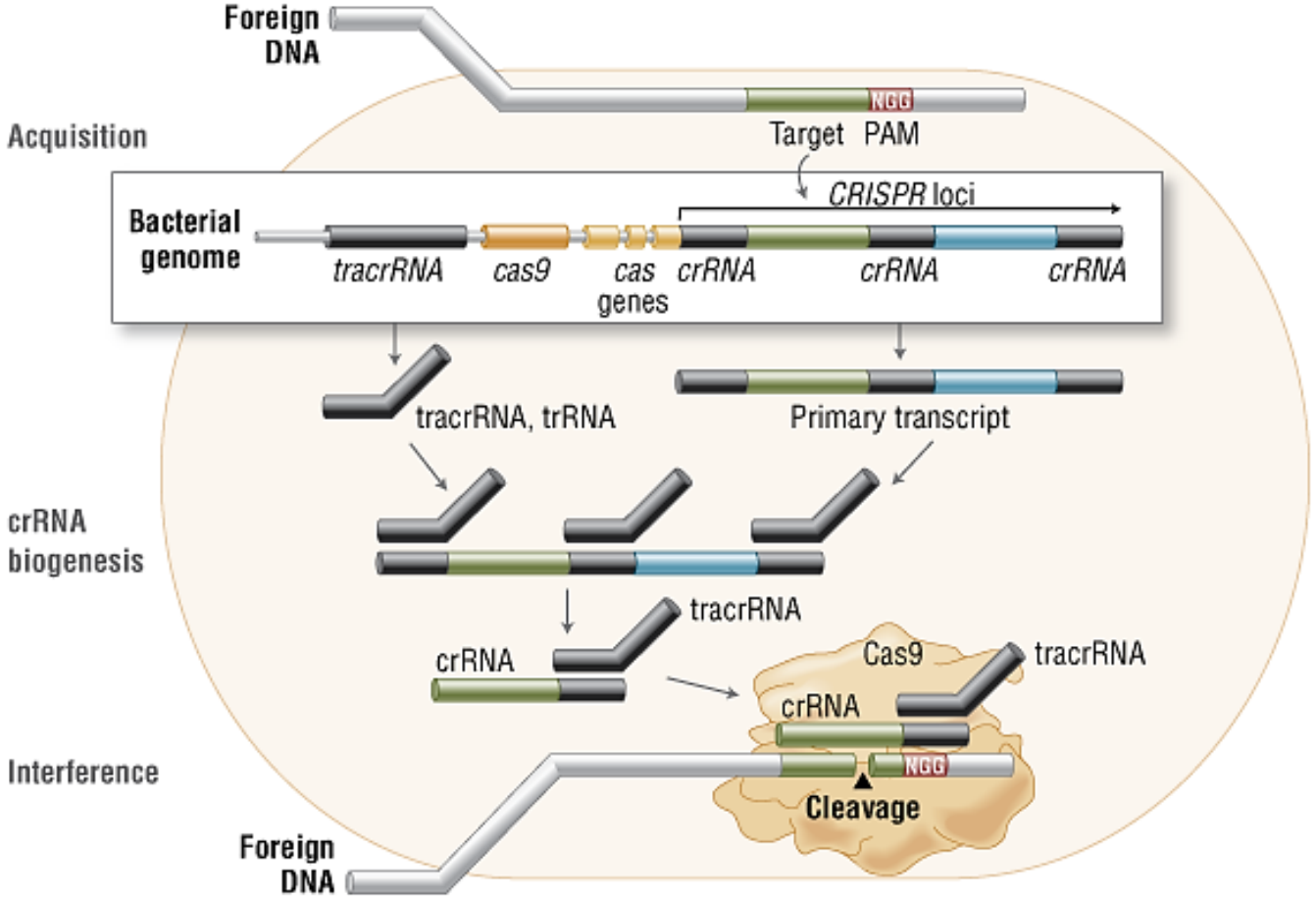
❑ A number of CRISPR repeats and spacers are transcribed into a single long transcript - pre-crRNA.

❑ 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.



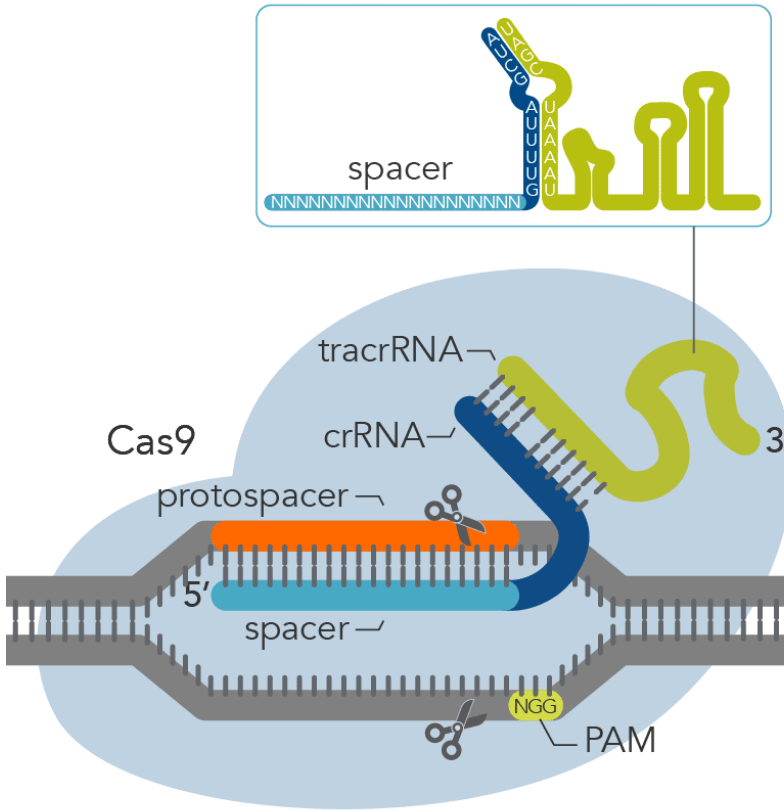


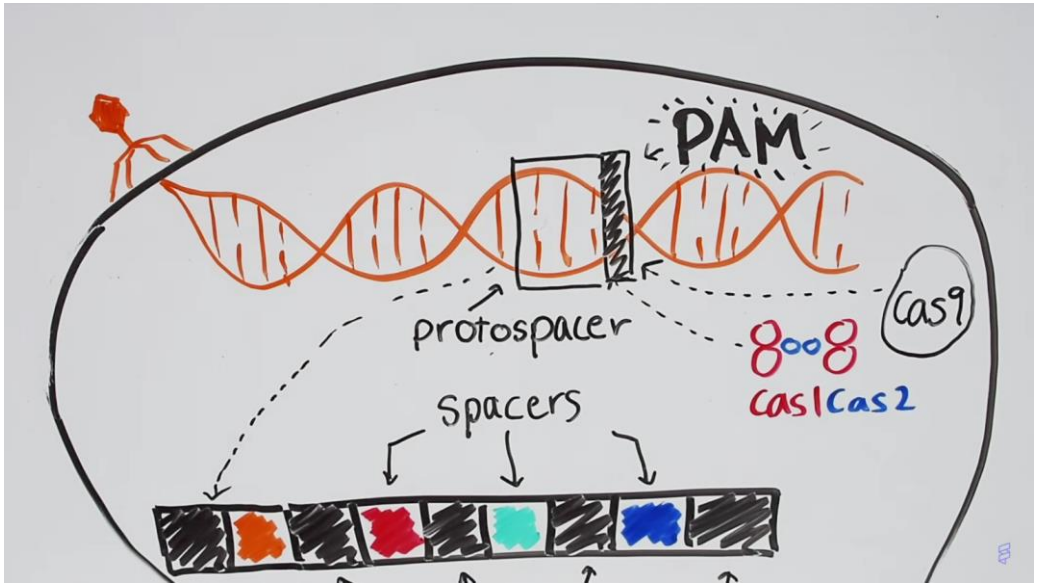
Interference

During the interference stage, crRNAs bind to their targets through base pairing and thus direct Cas endonucleases to cut and destroy the target.

The formation of a complex of crRNA and Cas proteins ensures the endonucleolytic destruction of nucleic acid sequences complementary to crRNA.

A prerequisite for such an action of the crRNA/tracrRNA/Cas nuclease complex is not only recognition of the target DNA by its complementary crRNA, but also the presence of a PAM region at the 3' end of the complementary DNA strand, which for the Cas9 system is the NGG sequence, where N is any nucleotide.



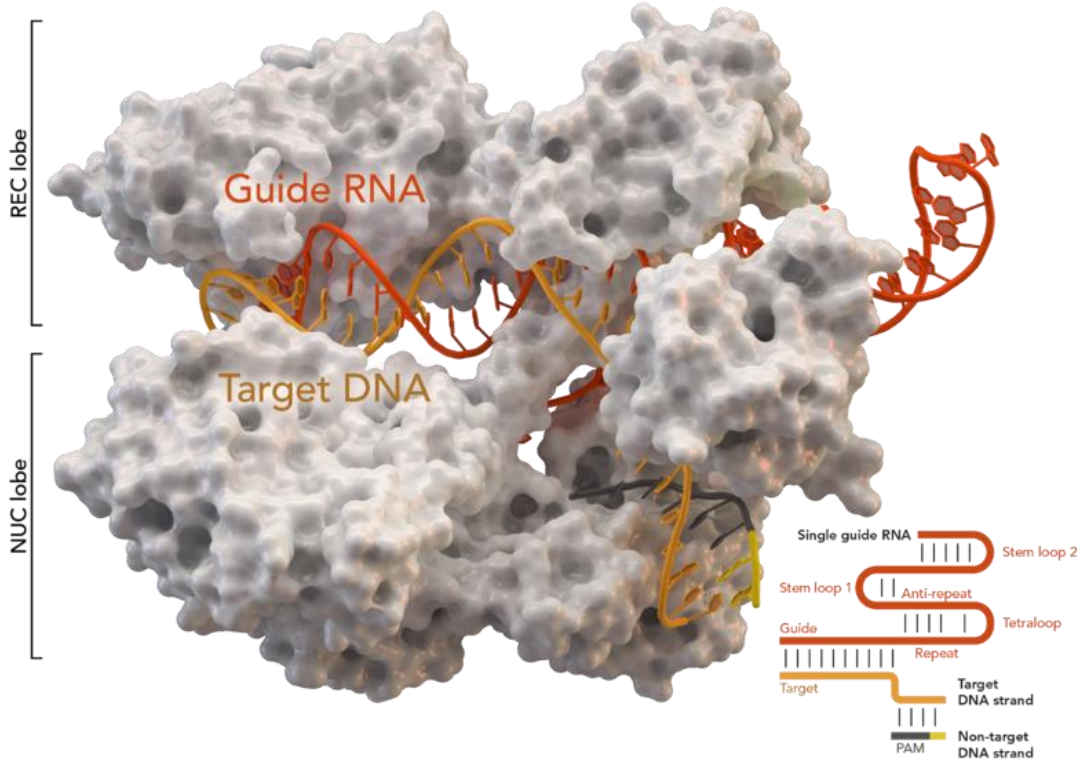


Cas9

Cas9 is a guide RNA endonuclease associated with the adaptive immune system CRISPR in a number of bacteria, particularly Streptococcus pyogenes. S. pyogenes uses Cas9 to store, inspect, and cut foreign DNA, such as bacteriophage or plasmid DNA.
Cas9 performs the check by unwinding foreign DNA and determining its complementarity with the twenty base-paired guide RNA spacer.

If the substrate is complementary to the guide RNA, Cas9 cleaves the foreign DNA. In this sense, the CRISPR-Cas9 mechanism has a number of parallels with the RNA interference (RNAi) mechanism in eukaryotes.

The safety of the practical application of this method is determined, among other things, by the fact whether the desired sequence of twenty paired bases is unique in the DNA being modified.



In 2012, Lithuanian biochemist Virginijus Siksnis was one of the first to demonstrate programmable DNA cleavage by one of the components of CRISPR-Cas systems, the Cas9 protein. Since 2007, the main direction of his research has been the study of the recently discovered CRISPR-Cas systems for protecting bacteria from bacteriophages and foreign genetic material.

According to Šikšnis, his article was not even recognized by a serious editorial board of an academic journal and was not sent to reviewers, so the time needed to be recognized as the first was lost.

Martin Schlack reported that Šikšnis presented his paper describing DNA cleavage by Cas9 in the peer-reviewed scientific journal Cell Reports on April 18, 2012.

After it was rejected without peer review, he submitted it to the peer-reviewed scientific journal PNAS a month later and took several months to review and publish.

Meanwhile, American biochemist Jennifer Daudna and French microbiologist Emmanuelle Charpentier published their paper in the peer-reviewed scientific journal Science, where it was reviewed and accepted within two weeks.

DELIVERY OF CRISPR/CAS REAGENTS TO PLANTS

The delivery of editing reagents to plant cells and the production of editing events are key steps in genome editing.

CRISPR-mediated editing reagents, including DNA, RNA, and ribonucleoproteins (RNPs), can be delivered into plant cells by protoplast transfection (injection), *Agrobacterium*-mediated transfer DNA (T-DNA) transformation, or particle bombardment.

Protoplast transfection is normally used for transient expression, whereas Agrobacterium-mediated transformation and particle bombardment are the two major delivery methods for the production of edited plants.

Such a drug, together with Cas9 and guide RNAs, should be easily reproducible enough for large-scale production of a drug to combat common diseases.

Unlike viral delivery systems, lipid nanoparticles meet these criteria.

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.

FUTURE PROSPECTS

Exploiting the Potential of Plant Synthetic Biology

Plant synthetic biology is an emerging f eld t hat combines plant biology with engineering principles t o design and produce n ew devices that exhibit predictable behaviors.

This fild will play an important role in traditional crop improvement and will enable the development of novel bioproduction processes.

Plants are the most important sources of the primary metabolites that feed the world (i.e., proteins, fatty acids, and carbohydrates), and they produce a diverse array of valuable secondary metabolites for medicinal and industrial purposes.

FUTURE PROSPECTS

Exploiting the Potential of Plant Synthetic Biology

Nitrogen is a critical limiting element for crop growth and development. Most nitrogen fixation (nif) genes and their relative expression levels have been characterized. To reduce our dependence on inorganic fertilizers, the CRISPR/Cas system could be used to transfer the genetic elements of the Nod factor signaling pathway from legumes to cereals such as wheat, allowing the cereal to fix atmospheric nitrogen.

In addition, an important goal of synthetic biology is to build regulatory circuits to manipulate plant behavior, producing novel traits that improve crop productivity.

FUTURE PROSPECTS

Exploiting the Potential of Plant Synthetic Biology

dCas9-mediated gene regulation via multiplex gene activation, repression, and epigenome editing offers unprecedented opportunities for designing synthetic transcription factors, which could be used to construct increasingly complex, programmable, efficient gene circuits.
For example, in the C4 rice project (160), it is difficult to guarantee that the C4 photosynthesis pathway installed in rice can efficiently fix carbon; fie-tuning gene expression in the C4 pathway is required to optimize protein levels to increase the efficiency of carbon fixation. CRISPR/Cas mediated multiplex gene regulation could serve as a tool for this synthetic biology project.

FUTURE PROSPECTS

Accelerating the Domestication of Wild Plants.

Modern crops have been selectively bred for thousands of years, leading to the introduction of important characteristics that enable mechanical harvesting of high-quality, nutrient-rich food.

However, this process has led to a loss of diversity that can affect fitness under certain environmental conditions.
example of crop domestication by genome editing is tomato.

Modern tomato cultivars derived from intensive inbreeding cycles are suffering from increasing biotic and abiotic stresses. Wild tomato plants that are naturally stress tolerant can serve as ideal materials for de novo domestication via precisely engineering the domestication genes.

FUTURE PROSPECTS

Improved Delivery Systems

The cell wall makes efficient delivery of genome-editing reagents to plant cells challenging. Current delivery systems are limited to specific plant species, genotypes, and tissues.

In addition, almost all the current methods require tissue culture, a long and laborious process. Improving the existing delivery systems and developing new systems will be key in reducing barriers to inexpensive application of gene editing in plants.

A further innovation will be achieving genotype-independent, tissue culture–free delivery via the plant germline or meristematic cells. Sperm cells, egg cells, and zygotes are emerging as realistic targets of delivery. For example, the use of pollen-mediated transformation would avoid the limitations of species specificity and regeneration using pollination or artificial hybridization.

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.

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

List of used literature:

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3. Ruud. Jansen, Jan. D. A. van Embden, Wim. Gaastra, Leo. M. Schouls. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Mol Microbiol*. **43**, 1565-1575;
4. Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, et. al.. (2015). CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein Cell*. **6**, 363-372;
5. Erik J. Sontheimer, Rodolphe Barrangou. (2015). The Bacterial Origins of the CRISPR Genome-Editing Revolution. *Human Gene Therapy*. **26**, 413-424;
6. https://hightech.plus/2018/07/25/crispr-dlya-chainikov-kratkii-putevoditel-po-glavnoi-bitehnologii
7. https://nplus1.ru/news/2019/11/19/ctx001-trial
8. https://www.skygen.com/podderzhka/obzory/29-redaktirovanie-genomov/

- **Home tasks:**

1. **History of discovery CRISPR/Cas genome editing tools.**
2. **CRISPR/Cas genome editing mechanism.**
3. **Applications for precision plant breeding and future prospects.**


GOOD LUCK!