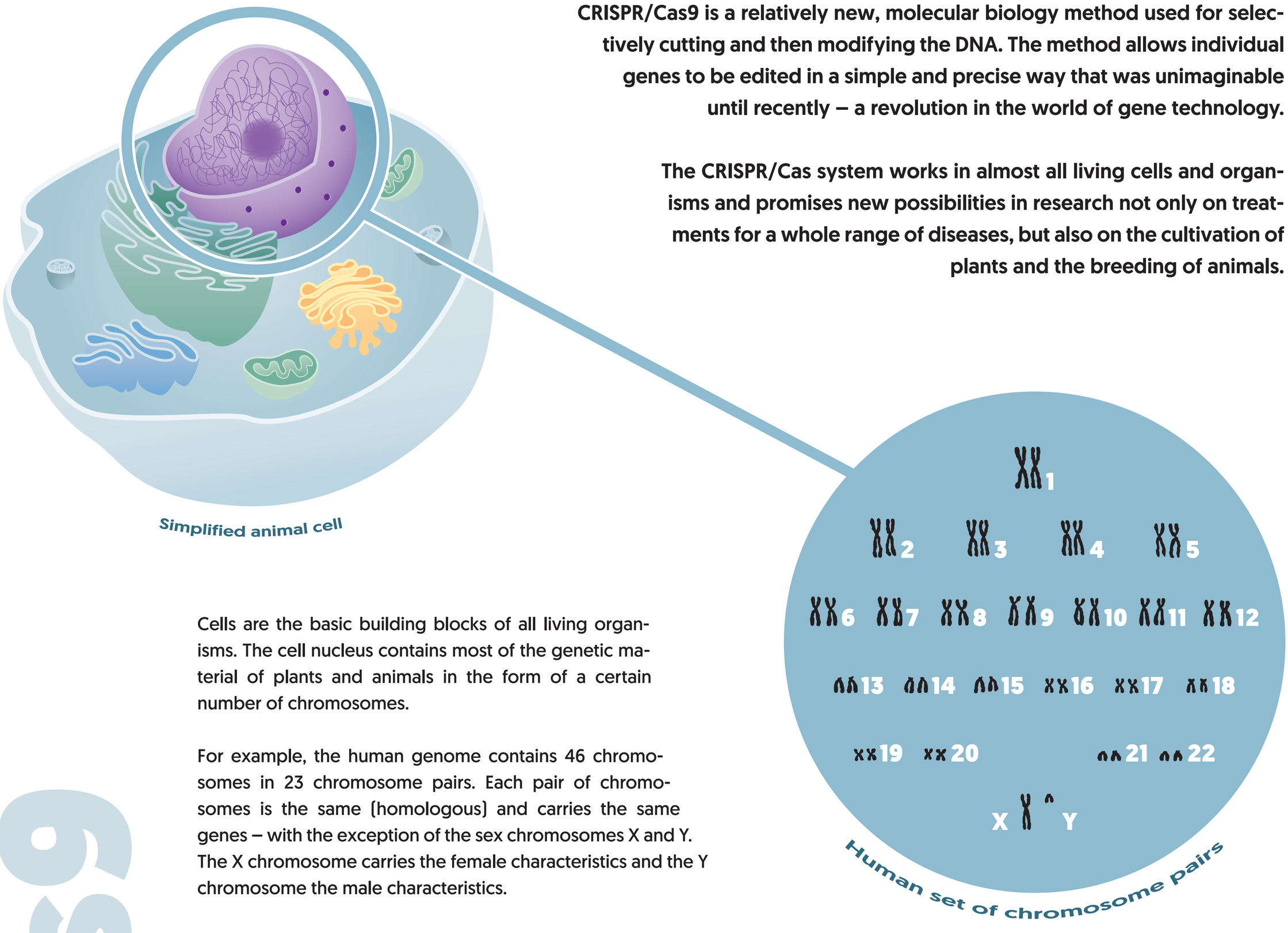
CRISPR/Cas9 is a relatively new, molecular biology method used for selectively cutting and then modifying the DNA. The method allows individual genes to be edited in a simple and precise way that was unimaginable until recently – a revolution in the world of gene technology.

The CRISPR/Cas system works in almost all living cells and organisms and promises new possibilities in research not only on treatments for a whole range of diseases, but also on the cultivation of plants and the breeding of animals.

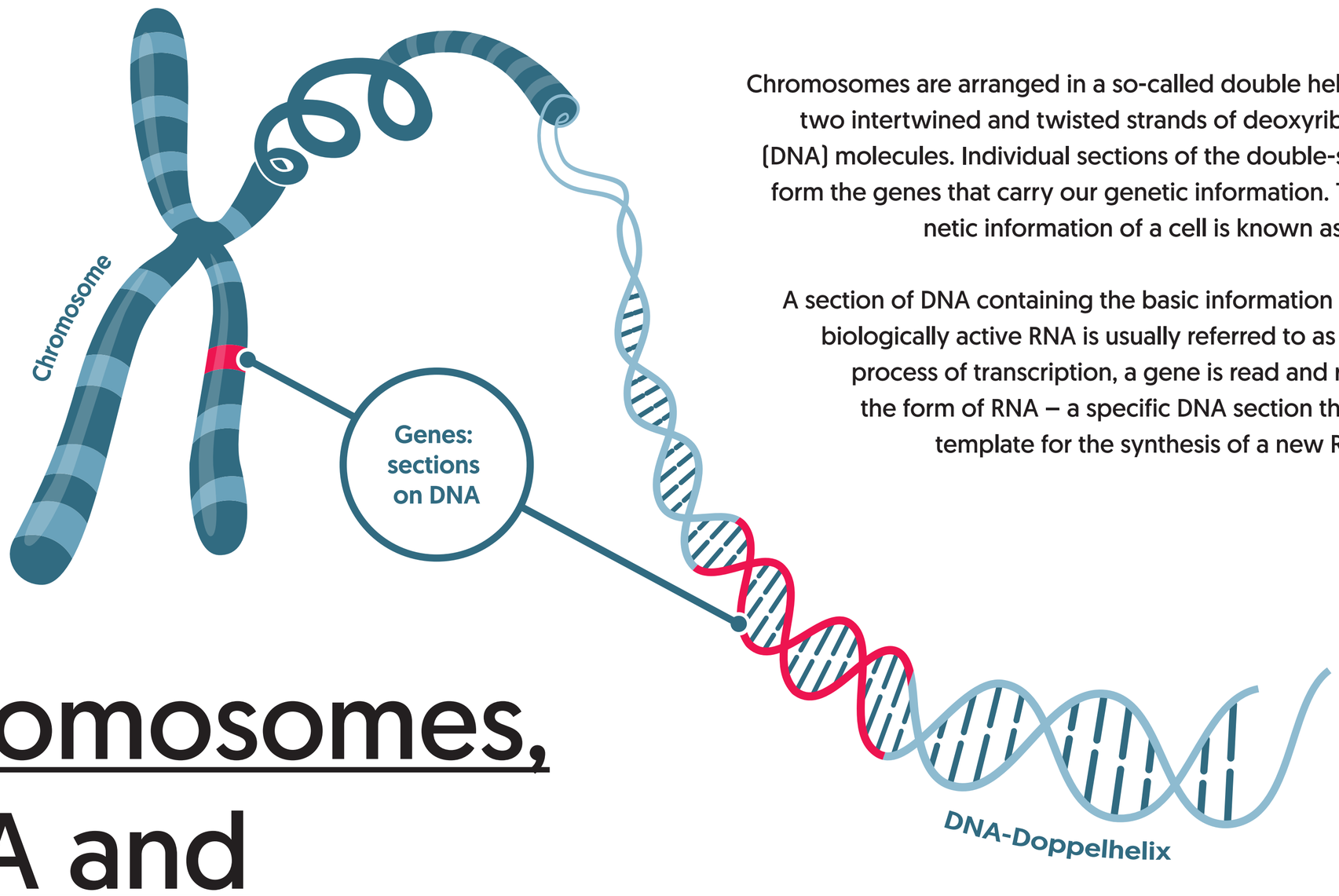


Simplified animal cell

Human set of chromosome pairs

Cells are the basic building blocks of all living organisms. The cell nucleus contains most of the genetic material of plants and animals in the form of a certain number of chromosomes.

For example, the human genome contains 46 chromosomes in 23 chromosome pairs. Each pair of chromosomes is the same (homologous) and carries the same genes – with the exception of the sex chromosomes X and Y. The X chromosome carries the female characteristics and the Y chromosome the male characteristics.



Chromosomes are arranged in a so-called double helix comprising two intertwined and twisted strands of deoxyribonucleic acid (DNA) molecules. Individual sections of the double-stranded DNA form the genes that carry our genetic information. The entire genetic information of a cell is known as the genome.

A section of DNA containing the basic information for producing biologically active RNA is usually referred to as a gene. In the process of transcription, a gene is read and reproduced in the form of RNA – a specific DNA section thus serves as a template for the synthesis of a new RNA molecule.

CRISPR/Cas9

Chromosomes, DNA and genes

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CRISPR/Cas9 in nature

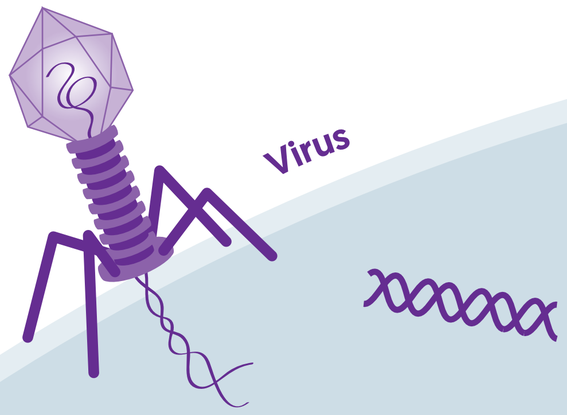
The CRISPR/Cas system originally comes from bacteria, where it serves as a kind of acquired immune system that can recognize and ward off attacks by viruses.

CRISPR is a family of repeating sections of DNA that occur in the genome of many bacteria and Archaea. When a virus attacks, parts of the foreign DNA are integrated in the CRISPR sections of the bacterial genome. The crRNA that is read off enables the immune system to recognize and destroy the virus DNA.

CRISPR:
Clustered Regularly Interspaced Short Palindromic Repeats

Cas:
Enzyme; molecular scissors

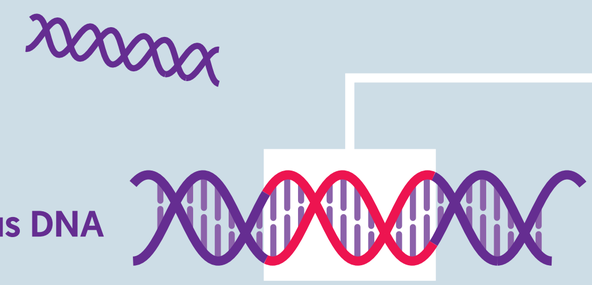
crRNA:
CRISPR-RNA; identifies the target sequence



Bacterium (cell)

1

Viruses attack a bacterium. They smuggle their DNA into the bacterium.

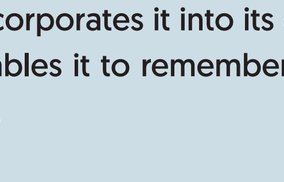


2

With the aid of enzymes, the bacterium cuts a section out of the virus DNA and incorporates it into its own (CRISPR) DNA. This trick enables it to remember the virus in case it attacks again.

3

This section is read off in the transcription process, giving rise to an RNA sequence known as CRISPR-RNA (crRNA).



crRNA

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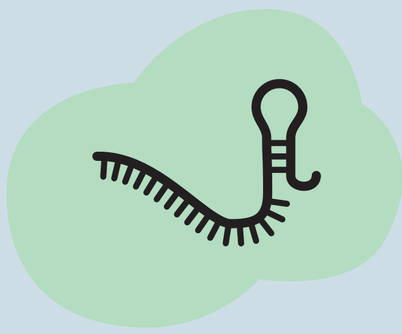

Cas-crRNA complex

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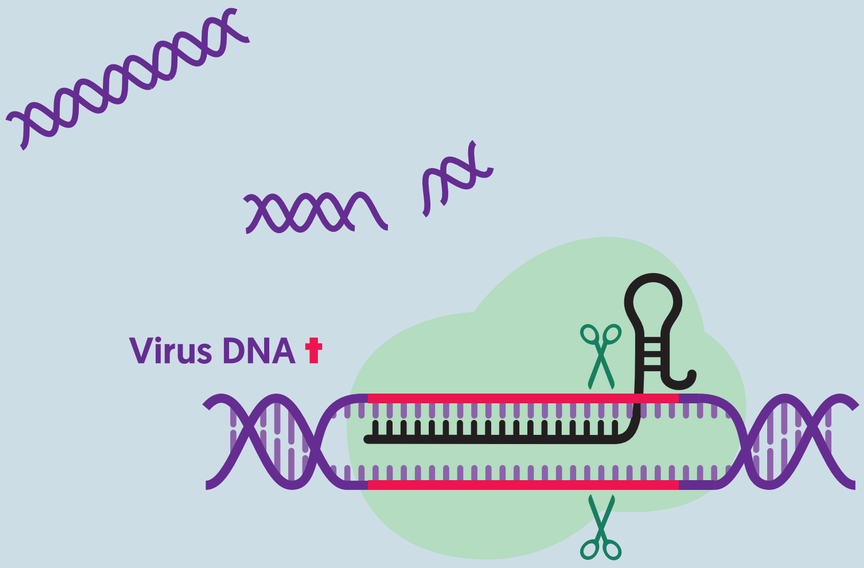
Cas9

4

The resulting crRNA forms a complex with the Cas enzyme. Thanks to its high degree of specificity, the crRNA acts like a molecular profile by which the matching foreign DNA can be identified.



5

The crRNA docks onto the recognized target sequence. The Cas enzyme can now cut the virus DNA at this point, rendering it harmless.

The DNA double-strand break is lethal for viruses, which are unable to repair their own DNA.

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The CRISPR/Cas method can also be used in a similar way in research to selectively cut and modify DNA (genome editing). In this way, genes can be inserted, removed or disabled.

There are three stages in this procedure: First, the exact point at which a modification is to be made must be found and selected. This target sequence is identified using a synthetically produced probe (lead RNA).

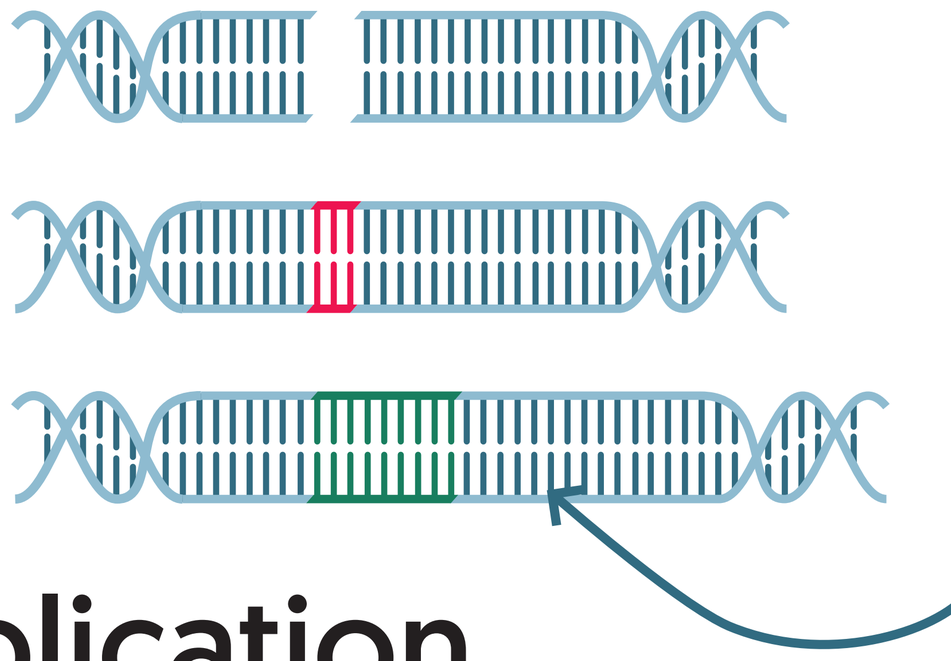
Lead RNA is produced in the laboratory and consists, amongst other things, of a matching crRNA.

It is essential that the sequence of the desired gene or DNA section is known. The crRNA forms the counterpart of the genes to be modified in a target DNA.

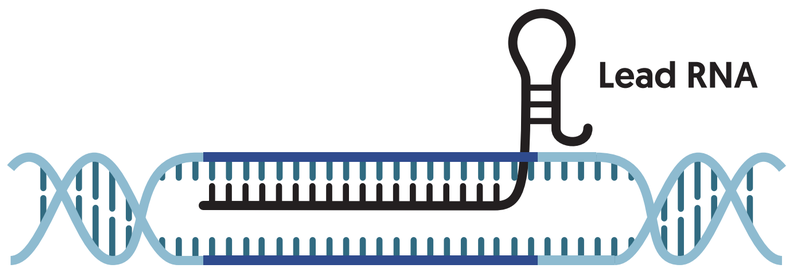
The lead RNA is coupled with the Cas9 enzyme, which acts as scissors, and is into the cell, e.g. by means of microinjection.

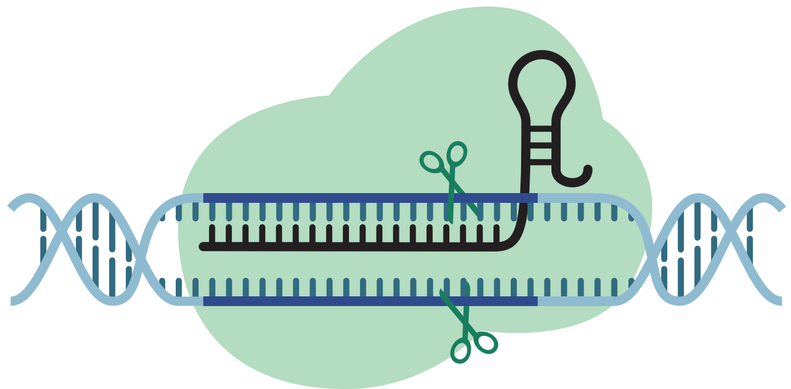
The lead RNA guides the CRISPR/Cas complex to the target (e.g. a gene), where the crRNA docks onto the desired site.

The Cas9 enzyme cuts the target DNA at the desired site.



The DNA double strand is then cut exactly at this point using the molecular scissors (Cas9 enzyme) linked to the RNA probes. This DNA break can then be repaired again and modified in different ways so that any changes inserted are stable and thus passed on to the next generation.





With the double-strand break, the cell's own repair systems then spring into action: they join the severed DNA strand back together again – but usually with minor errors. As a result of these mutations, it is no longer possible for the gene concerned to be read properly and so it is blocked.

Alternatively, individual DNA building blocks may be replaced or short sequences newly incorporated into the DNA strand.

By means of this mechanism, for example, genes that cause disease can be switched off or replaced.

CRISPR/Cas9 in science

Application

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While older gene manipulation techniques can always be unequivocally identified in retrospect as artificial interventions, gene editing by means of CRISPR/Cas9 is no longer distinguishable from natural mutation or breeding. The boundaries between natural and artificial mutation have become blurred. It is therefore unclear whether plants created using CRISPR/Cas9 are to be classified as genetically modified organisms (GMOs) or as cultures.

Risks

- Aside from the biological fundamentals for understanding genome editing, ethical issues are also important.
- Misuse or negligence could lead to the rapid spread of a few genetically modified organisms - including insects, for example. We therefore need international guidelines on the way research with this new instrument is conducted and where the limits should lie.
- With a new CRISPR sequence, it is possible that a very similar sequence in the DNA of other organisms could be modified by chance with fatal consequences.
- Until now, potentially dangerous manipulations were only possible in the best-equipped laboratories. This could change with CRISPR, because the technique does not require any complex equipment.
- CRISPR/Cas9 is too inefficient for germline manipulation and could potentially lead to dangerous side effects, i.e. if the CRISPR/Cas9 scissors cut the DNA at undesirable points. Since such rare effects could, for example, lead to the activation of cancer genes, scientists are working flat out to minimize such effects through improved enzymes.
- The combination of genome editing with Gene Drive approaches could result in certain genes being completely eliminated or also newly integrated in a population.

Opportunities

- CRISPR/Cas9 is simple, fast, precise, universal and cheap.
- Cell and animal-based models for the analysis of fundamental processes and diseases can now be generated much more quickly and in a greater diversity of species than ever before.
- The most promising strategy is based on isolating endogenous (stem) cells from patients and correcting their genetic defects by genome editing. Since repaired cells are only returned to the patient after a molecular review, it is hoped that the risk of adverse side effects can be minimized.
- The combination of genome editing with Gene Drive approaches could result in certain genes being completely eliminated or also newly integrated in a population.
- Plant DNA can be cut to shape using the molecular scissors so that they develop immunity to pests, allowing bacteria-resistant rice or mildew-resistant wheat to be produced.
- Treatment option in medicine:

  E.g. in HIV: The active CCR5 gene forms the basis for the spread of the human immunodeficiency virus via human immune cells (T cells). Researchers have therefore questioned whether HIV can be combated by switching off the intact CCR5 gene in the cells of infected patients. They are trying to achieve this using CRISPR/Cas9.

  E.g. in leukemia: An initial gene therapy with TALEN (earlier-generation molecular scissors) for the treatment of leukemia has already proved successful. T cells of a donor were genetically modified using TALEN.

  E.g. for donor organs: The lack of donor organs is driving research to find a solution to this problem, including the use of donor organs from pigs. Researchers are working on a solution that will simultaneously switch off all 62 viral genes in pig kidney cells that could potentially infect human cells.

Some risks, opportunities and side effects

CRISPR/Cas9

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