

CRISPR-Cas9: Mechanism and Applications

CRISPR-Cas9 is a type of immune system discovered in bacteria. Scientists have adapted its components into a biotechnology tool for editing DNA. Made up of a DNA-cutting enzyme (Cas9) and a programmable RNA molecule (guide RNA), CRISPR-Cas9 can be used to precisely target nearly any gene.

How it works

Step 1: Targeting

Scientists introduce the Cas9-guide RNA complex into a cell (in this case, a human cell), where it randomly associates and dissociates with the DNA. Cas9 recognizes and binds to a three-nucleotide sequence motif called PAM that is abundant throughout the genome.

One way to think of the Cas9-guide RNA complex is as a molecular scissor (Cas9) with a programmable GPS (guide RNA).

Cas9 is a nuclease, a type of enzyme that cleaves DNA. It recognizes and binds to a three-nucleotide sequence motif called PAM that is ubiquitous throughout a cell's genome (the complete set of genetic material present in a cell or organism).

Scientists synthesize the guide RNA to contain a 20-nucleotide sequence that matches a particular sequence in a cell's DNA that they want to target. When the guide RNA is added to Cas9, it will guide Cas9 to this target sequence. The target sequence can be nearly any sequence as long as it occurs near a PAM motif; it can be part of a gene's coding region or a regulatory sequence that scientists want to change in some way.

Definitions

Cellular DNA: The genetic material of an organism stored in the nucleus of cells. It consists of long sequences of nucleotides.

Cas9: CRISPR stands for clustered regularly interspaced short palindromic repeats, which are repeating sequences found in the DNA genome of bacteria. Cas9 stands for CRISPR associated protein 9. It is an endonuclease, meaning it's an enzyme that cuts nucleic acids.

Guide RNA: A sequence of RNA that is synthesized to match a target sequence of interest, such as a sequence within a particular gene.

PAM: PAM is short for proto-spacer adjacent motif. It is usually a three-nucleotide sequence consisting of 5 prime-NGG-three prime in which the N represents any nucleotide (A, C, G, or T) followed by two guanine (G) nucleotide bases. In humans, PAM motifs occur approximately every 50 bases or less, which explains why you can use the Cas9-guide RNA complex to target nearly any human gene.

Step 2: Binding

Once it binds to a PAM motif, Cas9 unwinds the DNA double helix. If the DNA at that location perfectly matches a 20-nucleotide sequence within the guide RNA, the DNA and matching RNA will bind through complementary base pairing.

Cas9 recognizes and binds to PAM motifs in the cell's DNA. The motif consists of any nucleotide (designated "N") followed by two guanines, when looking at a DNA sequence in a 5 prime to 3 prime direction (N-G-G). This

sequence motif is abundant throughout the human genome. After binding, Cas9 unwinds and pulls apart the DNA double helix upstream of PAM—in other words, closer to the 5 prime end of the DNA strand relative to PAM.

If the sequence of the unpaired DNA strand is not an exact match to the 20-nucleotide sequence within the guide RNA, Cas9 disengages from the DNA, which zips back up into a double helix. If the sequences are a perfect match, the guide RNA base pairs with the complementary DNA sequence, forming a DNA-RNA helix.

Definitions

Target DNA: A sequence of DNA that matches a 20-nucleotide sequence in the guide RNA and will be targeted by the Cas9 nuclease.

Cas9 PAM-interacting domain: The region of the Cas9 protein that recognizes and binds to the PAM sequence motif.

Step 3: Cleaving

The DNA-RNA pairing triggers Cas9 to change its three-dimensional structure and activates its nuclease activity. Cas9 cleaves both DNA strands at a site upstream of PAM.

When the guide RNA perfectly aligns with the target DNA, the RNA and DNA will form a DNA-RNA helix. This binding event activates Cas9's nuclease, or DNA-cutting, activity. It makes specific cuts in the DNA at a position three nucleotides upstream from the PAM site. Two active sites (regions where molecules bind to undergo chemical reactions) on the nuclease domain of Cas9 generate the cuts and cleave both strands of the DNA double helix, resulting in a double-stranded DNA break.

Definitions

Cleavage site: The position where the DNA strand is cut by Cas9, typically three nucleotides upstream of the PAM site.

Cas9 nuclease domain: The region of the Cas9 protein that cleaves DNA. Two different sites within the nuclease domain make the cuts, one on each DNA strand, resulting in a double-stranded DNA break.

Step 4: DNA Repair

Cells contain enzymes that repair double-stranded DNA breaks. The repair process is naturally error-prone and will lead to mutations that may inactivate a gene. Cleaving DNA at a precise location is one of many applications of the CRISPR-Cas9 technology.

Definitions

Double-stranded DNA break: Both strands of the DNA helix are cut. It can lead to mutations or genome rearrangements if the DNA strands are not rejoined correctly.

Repaired DNA Sequence: The DNA sequence after the cell machinery repairs the Cas9-induced break. The sequence is very similar to the original sequence but the repair process can result in mutations.

Mutation: A change in a DNA sequence.

NON-HOMOLOGOUS END-JOINING

CRISPR-induced double-stranded DNA breaks can be repaired by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR).

NHEJ is the more frequently used, faster repair mechanism, because the cell does not use a template to join broken DNA ends together. It is, however, an error-prone process that can introduce mutations in the target sequence. Errors are rare, but when the break is repaired correctly, Cas9 will once again recognize the target sequence and cleave it. Repeated cycles of cleavage and repair eventually result in a mutation. The type of mutation is random, but it will occur precisely within the desired target sequence. If the target sequence is within a gene's coding region, the mutation will likely inactivate that gene.

Definitions

Gene inactivation: Mutations that inactivate a gene typically turn off its expression so that no protein is produced or result in a nonfunctioning protein.

HOMOLOGY-DIRECTED REPAIR

The second type of repair mechanism is homology-directed repair (HDR), which is less error-prone and uses a homologous DNA template to accurately repair the break (for example, from a sister chromatid). Scientists can manipulate this repair system by introducing into the cell an excess of a DNA repair template along with the Cas9-guide RNA complex. The cell's repair machinery will be "tricked" into using the repair template to fix the break by HDR. By designing different repair templates, scientists can change the target DNA sequence into a new sequence. These templates could also correct an existing mutation by replacing it with a non-mutated sequence of DNA.

Definitions

Gene editing: A genetic engineering technique for making specific changes in the DNA of a cell or organism. DNA sequences can be inserted, modified, or deleted.

DNA repair template: A sequence of DNA that provides the homology necessary for the repair of a double-stranded break. Scientists can add a repair template to a cell to insert a new DNA sequence at the site of the break.

Cleaved DNA: DNA that has been cut on both strands of the double helix, causing a double-stranded break.

New DNA sequence: The repair template contains a new sequence flanked by segments of DNA homologous to the ends of the cleaved DNA so that it can be used in the repair process.

About CRISPR-Cas9: Mechanism and Applications

You may have heard of CRISPR or CRISPR-Cas9. The name comes from the CRISPR system in bacteria, where it was first discovered. There, it functions as a type of immune system. Scientists have modified the bacterial system to produce a biotechnology tool for editing the DNA of different cells and many other applications. Explore this dynamic interactive to learn the basic principles of how this revolutionary tool works and the many ways in which scientists are using it in their research.

The How It Works view shows the molecular details of the most common application of the CRISPR-Cas9 tool: cleaving and editing DNA at a precise location, using a human cell as an example. The How It's Used view consists of short interviews with scientists who are using this tool in a variety of ways, not just to inactivate or edit genes, but also to turn on gene expression, label DNA, or even change the chemistry of specific nucleotides.

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Educator Resources

Coming soon

Help

Navigate through the interactive by scrolling, selecting the nodes on the navigation interface on the left, and/or using the Next and Previous navigation buttons at the bottom of the page. Select the pink pulsating plus signs to learn more about each step in the process. Learn about the mechanism of action by clicking on How It Works and hear how scientists are using CRISPR in their research by clicking on How It's Used.

Browser and device support

This interactive is optimized for use on desktop as well as modern tablets and smartphones. The application is supported by the most-recent versions of Google Chrome, Firefox, Microsoft Edge and Safari web browsers. Note that users have reported issues with smoothness of the *How It Works* animation in Safari. For the best experience, we recommend the Google Chrome web browser.

Feedback

For feedback and suggestions about the Click and Learn, email biointeractive@hhmi.org