Biotech In focus

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Genome Editing: Brave New Tools

Our recent bulletins described RNA interference (RNAi), a powerful method for "silencing" genes. This approach prevents the genes from making proteins, and thus reducing their impacts on plants. In this installment, we describe another recent innovation that may have even greater implications for biotechnology.

Decades before we learned in detail that genes are specific sequences of the four chemical "letters" in DNA, scientists were altering genes. The objective was to study the genes effects on organisms. Initially, this work was limited to causing mutations by treating an organism with irradiation and chemicals. These mutations that changed the DNA sequences were passed from one generation to the next and scientists observed what went wrong, or right, in the mutant offspring. More recently, methods were developed to add new genes, first at random locations and then at specific sites (DNA sequences) in the genome. The genome is a cell's full set of DNA, that is divided into one or more chromosomes. Scientists made rapid advances in animal gene targeting, learning to delete, change, or replace specific genes in mice and later in other animals. Only in this century have researchers been able to target plant genes.

In the first gene targeting methods that work well in plants, a DNA-cutting enzyme was joined to combinations of small proteins that bind to specific DNA "letters" (nucleotides). After the cutting enzyme broke the plant DNA at the targeted (specific) site, the plant attempts to repair the damage. If no template or nucleotide pattern for repair was available, the broken DNA ends are joined back together. However this joining process tended to create mutations by putting the nucleotodes in the wrong order, so that the targeted gene no longer worked. The effects of losing that gene can then be studied.

However, if researchers provide a template gene whose ends match and paired with DNA sequences on either side of the break, a different form of DNA repair then can replace the broken gene, based the template of nucleotides. The replacement gene template can be entirely different from the broken gene, or it can differ by only one nucleotide. This means that we can now edit the instructions for most forms of life, cutting and pasting genes with relative ease.

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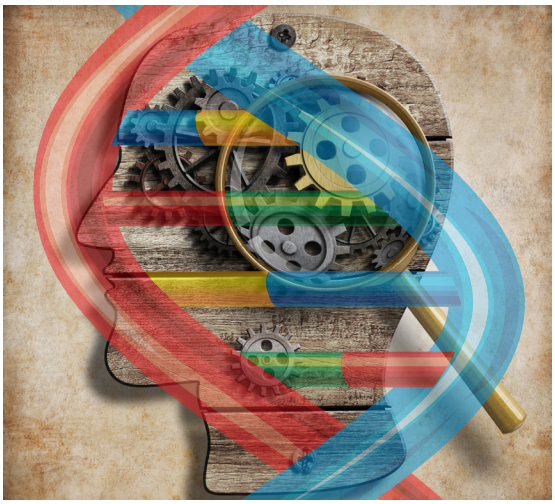
CRISPR – A Memory Bank

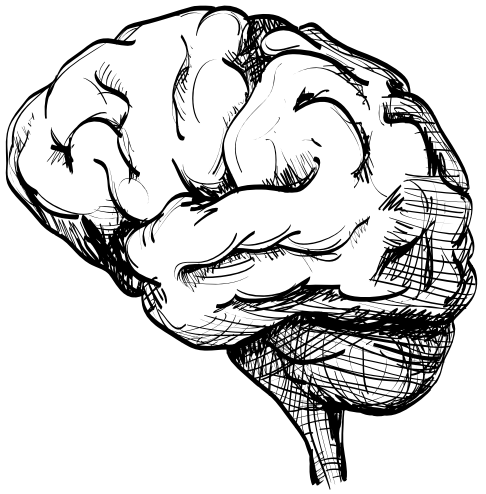
The possibility of editing human DNA raises important philosophical and ethics questions, while in the context of plant breeding, genome editing continues the process that began with earlier forms of genetic engineering, that allowed scientists to add, subtract, or change crop traits with increasing precision.



Not long before our first Biotech in Focus bulletin, a method was invented that made genome editing simpler—and much, much cheaper. This new tool takes its name from a bacterial DNA sequence called CRISPR, for Clustered Regularly Interspaced Short Palindromic Repeats. We can visualize CRISPR as a "necklace" in which short, symmetrical patterns of black-and-white beads (the repeats), are separated by stretches of colored beads (the spacers). The function of CRISPR in bacteria was a mystery until DNA sequencing revealed that the spacers contain viral DNA. In the same way that our immune system stores a memory bank of antibodies—proteins that bind to disease-causing organisms and target them for destruction—the bacterium uses DNA sequences from viruses that have attacked it (or its ancestors) in the past to make virus-binding RNAs within its own genome. The CRISPR sequence is the memory bank of the bacterium's immune system.

The Brains

Also essential to this defense mechanism are bacterial proteins that are produced from CRISPR-associated (Cas) genes located near the CRISPR sequence. We can think of the CRISPR sequence memory bank as the brains of CRISPR-Cas and the Cas proteins as the virus-fighting "brains".



Hijacking DNA

A virus injects its DNA into a bacterial cell, hijacking the cell to make copies of the virus. In response, Cas proteins chop up pieces of viral DNA and add new spacer segments to the CRISPR sequence. The cell then makes it's own "immune system" by in turn hijacking parts of the viruse's DNA! This process is managed by the CRISPR system.

Generating Traits

Similar to the RNAi mechanism present in most plants, animals, and fungi, the bacterial CRISPR-Cas system combines virus-targeting specificity provided by RNA and virus-killing digestive power provided by host-cell proteins. Components of the CRISPR-Cas system have been harnessed by biologists, including plant scientists, to conduct basic research on gene function and to generate new traits in plants. We'll discuss some of these CRISPR-Cas applications in our next bulletin.

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