The discovery of the CRISPR/Cas system has proven to be a ground-breaking technology for easily and precisely modifying DNA. CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is an “adaptive immune system” found in bacteria that confers resistance to foreign genetic elements, such as plasmids and phages (Figure 1). When bacteria are exposed to an invading organism, the bacterial “immune system” cuts pieces of the invader’s DNA and inserts these pieces as “spacers” into the CRISPR locus of the bacterial DNA. As a result, the bacteria build a library of DNA fingerprints specific to each experienced infection. These “spacers” specify the exact site in an invader’s DNA that will be targeted for cutting or splicing.

Associated with the CRISPR locus are a set of genes—called Cas, for CRISPR-associated genes. Cas genes code for proteins crucial for the cutting and splicing of foreign DNA. In Streptococcus pyogenes, the Cas 9 gene encodes for a non-specific double-stranded DNA endonuclease.

The transcription of the “spacers” along with the Cas genes allows for targeting and splicing the foreign DNA, making this system a powerful genome editing tool.

**Figure 1.** Mechanism of the CRISPR system.
engineering technology. The RNA template made from the “spacers,” which is called crRNA, specifies the foreign DNA to be subsequently targeted via complementary base pairing. The Cas protein nuclease cleaves the foreign DNA, thereby preventing infection of the host.

In nature, targeting the invading DNA occurs in a few key steps. First, the “spacer” DNA is transcribed to crRNA. Additionally, the Cas genes are transcribed and translated into Cas proteins. Next, a trans-activating crRNA, called tracrRNA, associates with the Cas protein and the crRNA. Finally, this Cas-crRNA-tracrRNA complex can target DNA for cleavage at the site specified by the crRNA.

In a laboratory, specific crRNAs and the tracrRNA can be linked together as a “guide RNA,” or “gRNA strand” for greater efficiency in the system. One can change the specific DNA sequence that is targeted simply by changing the crRNA portion of the gRNA. There are commercially available RNA sequences to be used for targeting.

Importantly, for the Cas:gRNA complex to successfully target DNA, the target DNA must be adjacent to a two-base-pair sequence called a protospacer adjacent motif (PAM). Once the target DNA has been spliced, the cell can repair the DNA break on its own or repair DNA can be provided for insertion at the site.

The clinical relevance of the CRISPR/Cas system is substantial. CrRNA can be engineered to target a specific site on the genome of an organism for removal or modification. Therefore, this technology allows us to target and remove a mutated gene and insert the normal gene in the laboratory and potentially in the clinic. The decision to supply the repair DNA or to allow the cell to repair the DNA break on its own would depend on the clinical application.

Additionally, the Cas protein can be used for transcriptional control of a target gene by removing the Cas protein’s nuclease activity while retaining its DNA-binding ability. This “catalytically inactive” Cas can repress or “knock-out” transcription of a target gene. This technique has been used to create knock-out animal models of retinitis pigmentosa and other retinal dystrophies.

The potential for CRISPR/Cas to cure hereditary ophthalmic diseases, such as retinitis pigmentosa, hereditary optic neuropathies, and hereditary maculopathies, by gene replacement is clear. Targeted gene therapy may also be useful for both wet and dry forms of macular degeneration. Using the CRISPR/Cas genome editing technology, scientists have succeeded in replacing defective genes in models of several diseases, including retinitis pigmentosa, muscular dystrophy, and neuro-degenerative diseases.

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