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# CRISPR: The Last Piece of the Genetic Puzzle

Brad Broyles

**Abstract:** CRISPR/Cas9 is an emerging gene editing tool. This technology, which evolved naturally as a defense mechanism for bacteria and other prokaryotes, has been repurposed to edit genes in a wide array of cells, including human cells. This paper outlines the discovery, mechanism, and potential applications of CRISPR/Cas9.

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Most physical harm arises in three ways: injuries, infections, and genetic diseases. For the most part, injuries and infections are well understood and treatments well established. Genetic diseases are more difficult to understand, and treatments usually target the symptoms, not the cause of the disease. Prevention revolves around removing environmental contributors to the disease, but it fails to address the genetic aspect of the disease. Finding effective ways to treat and prevent genetic diseases is the last puzzle piece in ensuring physical wellness. While there are many emerging genetic therapies, one of the most promising is the gene editing CRISPR/Cas9 technology.<sup>1</sup>

What are CRISPR and Cas9? In 1987, researchers studying the bacteria *E. coli* noticed short repeated segments in the bacteria's DNA. The repeated segments were grouped near each other on the bacteria's chromosome separated by unique DNA sequences called spacers.<sup>2</sup> This group of repeated segments and spacers is called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). In addition to the short repeats and spacers, there are other genes and proteins that work with CRISPR. The other genes and proteins that work with CRISPR are said to be CRISPR-associated (Cas). Since its discovery in 1987,<sup>3</sup> CRISPR has been identified in around 50% of bacteria.<sup>4</sup> In 2005, it was noticed that these unique spacers matched foreign DNA fragments from viruses and plasmids that infect bacteria. It was predicted that CRISPR could possibly be a way for bacteria to protect themselves from foreign DNA.<sup>5</sup> In 2008, it was proven that CRISPR could provide protection from foreign DNA.<sup>3</sup>

The way CRISPR provides protection for bacteria can be summarized into three phases: adaption, biogenesis, and interference. In the adaptation phase, foreign DNA enters the bacteria, and a part of this DNA, known as the protospacer, is cut out and added to the CRISPR region of the bacteria's chromosome as a new spacer.<sup>6</sup> This process allows the bacteria to keep a record of the viruses or other foreign DNA by which it has been infected. The way CRISPR integrates these new spacers into the genome or selects the protospacer from the rest of the foreign DNA is known for some bacteria and unknown for others.<sup>7</sup> The biogenesis phase occurs when the portion of this foreign genome that was previously stored as a spacer in the CRISPR region is copied into CRISPR RNA (crRNA). The last phase, interference, occurs when a Cas protein uses the crRNA as a template to match the foreign DNA.<sup>6</sup> The outcome following the crRNA

and DNA match is dependent upon which Cas protein was utilized. For example, Cas3 cuts and degrades single stranded DNA; Cas10 degrades both DNA and RNA; and Cas9 cuts double stranded DNA.<sup>6</sup> By cutting and degrading the foreign genome, CRISPR effectively protects the bacteria from recurring infections.

It is clear CRISPR can help bacteria survive, but how can employing this system be useful in humans? Interestingly, the same mechanisms the bacteria use to prevent infections can also be used to cut other sequences of DNA, including human DNA. By changing the crRNA guide template associated with the Cas9 protein, it could be possible to edit any gene in the human body!<sup>5</sup> CRISPR/Cas9 refers to the use of crRNA and the Cas9 protein to edit genes. CRISPR/Cas9 as a gene editing tool works almost in the same way as CRISPR interference. The Cas9 protein, with the help of a guide crRNA sequence, binds and cuts double stranded DNA.<sup>8</sup> However, instead of degrading the DNA like CRISPR interference in bacteria cells, human cells attempt to repair the damaged DNA. The cell can utilize two different repair pathways: non-homologous end joining and homology-directed repair. Non-homologous end joining (NHEJ) does not always rejoin the cut DNA perfectly, and that can often result in a mutated gene.<sup>6</sup> While normally a gene mutation would be detrimental, it could be useful in gene editing to selectively turn off an unwanted gene.<sup>9</sup> Homology-directed repair (HDR) does not reattach the broken DNA; instead it inserts new DNA into the break that is homologous, or similar, to the original sequence. When HDR takes place, there is the possibility a new gene will be inserted into the genome.<sup>10</sup> This activity can replace non-functional genes with functional genes.

It is also possible to use CRISPR/Cas9 to affect gene expression without cutting the DNA. This process requires a Cas9 protein that can bind to the DNA but lacks cutting ability.<sup>6</sup> The mutated Cas9 remains on the DNA and makes transcription of the gene more difficult. Transcription is an important step in expressing genes, so by making it more difficult, the expression of this gene is reduced and even silenced in some genes.<sup>11</sup> Mutated Cas9 proteins can also bind to silencers, which are places that reduce gene expression. When bound, Cas9 proteins prevent the action of silencers and increase transcription of a certain gene.<sup>12</sup> Ideally, CRISPR/Cas9 could be used to replace or remove any gene and increase or decrease any gene's expression.

However, problems may occur in the body when using CRISPR/Cas9. Off-target mutations are one concern associated with CRISPR/Cas9 use.<sup>13</sup> The Cas9 protein may cut the DNA somewhere other than its intended target, which can cause an unwanted and potentially harmful mutation. Another problem is delivering the Cas9 protein and the guide crRNA into the cells.<sup>14</sup> However, CRISPR/Cas9 is a new technology, and new systems are being developed to overcome these challenges. Synthetic CRISPR RNA (scrRNA) uses chemically modified nucleotides to work around the delivery problem while also managing to increase cleavage activity and decrease off-site mutations.<sup>15</sup> Despite the achievements of new CRISPR/Cas9 systems, their application and use still raise concerns. Until CRISPR/Cas9 is better understood, parts of the scientific community are requesting moratorium on all studies that would use CRISPR/Cas9 to alter cells that pass genes on to children.<sup>16</sup> Studies that use CRISPR/Cas9 to edit cells that do not pass genetic information on to children are still being conducted.

One developing area of research for CRISPR/Cas9 is cancer treatment. Cancer is a disease that affects millions of people around the world. One approach for cancer treatment is to fix tumor suppressor genes.<sup>16</sup> In a cancer cell, tumor suppressor genes are mutated. CRISPR/Cas9 has been used successfully to activate multiple tumor suppressor genes in bladder cancer cells. These activated tumor suppressor genes were shown to induce apoptosis or cell death, inhibit growth, and inhibit metastasis of the bladder cancer cells.<sup>17</sup> CRISPR/Cas9 was also used to knock-out the CDK11 gene, which promotes cancer cell proliferation in bone cancer. Silencing the CDK11 gene led to a decrease in bone cancer cell growth, and even induced apoptosis for some bone cancers.<sup>18</sup> Treating and preventing cancer with CRISPR/Cas9 is an exciting new field that will hopefully expand in the near future.

Another potential application for CRISPR/Cas9 is the treatment and prevention of human acquired immunodeficiency syndrome (AIDS). AIDS is the result of an infection from the human immunodeficiency virus (HIV). CRISPR/Cas9 can be used to treat AIDS in multiple ways. One way is to attack and cut out latent viruses that have incorporated into the host cells genome. The virus most often targets Helper T cells, where it integrates its own genome into the host cell.<sup>19</sup> CRISPR/Cas9 is being used to program the crRNA guide for the Cas9 protein so it matches up with the latent viral DNA in the Helper T cell. When the CRISPR/Cas9 system is in the Helper T cell, it cuts and mutates the latent viral DNA, so the virus can no longer replicate and infect other cells.<sup>20</sup> This kind of treatment is called a “sterilizing cure” because it rids the body of all of the viruses, even latent strands. There are also “functional cures” where the latent virus is still present, but new infections are prevented.<sup>21</sup> Research is being conducted on a “functional cure” that mutates a receptor HIV needs for binding to the Helper T Cells.<sup>19</sup> CRISPR/Cas9 can be used to mutate this CCR5 receptor to a naturally occurring mutated version, CCRΔ32, that is resistant to HIV infections.<sup>22</sup> Although the use of CRISPR/Cas9 for treatment and prevention of HIV/AIDS is in its early stages, it looks promising and could potentially cure a devastating disease.

It is possible that the different applications of the CRISPR/Cas9 system will see growth in the coming years. Interest in CRISPR/Cas9 has only increased since its discovery, and the favorable study results in treating HIV and cancer hopefully increase interest in these areas. Successfully treating and preventing diseases by addressing the genetic aspect via CRISPR/Cas9 therapy could help to complete the puzzle and improve physical wellness for people across the globe.

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