What Happens After the Cut

Introduction

CRISPR Cas9 is a remarkable protein that can be programmed with a guide RNA to seek out a statistically unique sequence in the 3.2 billion base-pair human genome and make a double-stranded cut at that site.

But that is all that Cas9 does. Mind you, that is a lot, and it is the first step in editing the human genome. But after making a double-stranded cut in DNA, Cas9 does not actually participate in the subsequent steps that can result in an edited gene. This brings us to the inevitable question. . . ***What happens after the cut?***

What do you think might happen to DNA after it is cut by the Cas9 protein?

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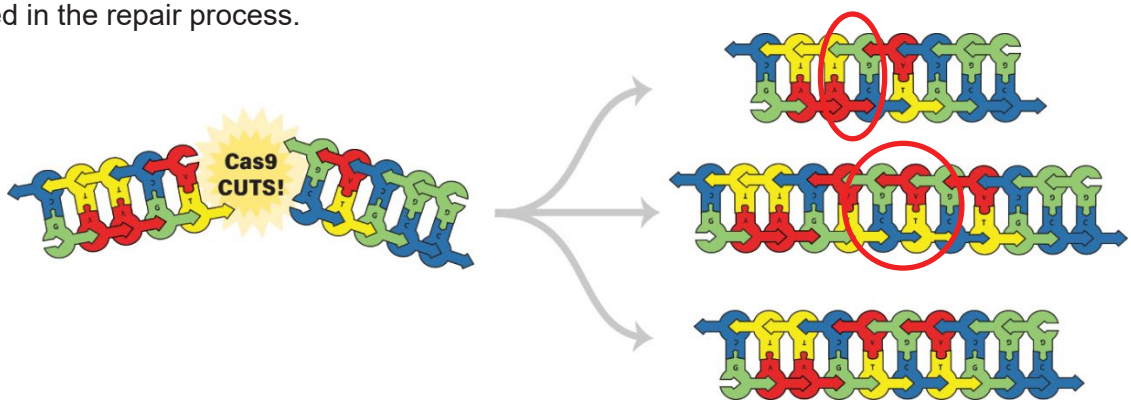
Attempting Repair

A double-stranded cut in genomic DNA is a potentially lethal event for the cell. To avoid the calamity that would result from a break in its DNA, eukaryotic organisms have evolved several different (redundant) DNA repair systems to deal with this problem. These two repair systems are known as ***Non-Homologous End Joining (NHEJ)*** and ***Homology Directed Repair (HDR)***.

Non-Homologous End-Joining (NHEJ)

The most active repair system is known as ***Non-Homologous End-Joining*** (NHEJ). It is an error-prone system that is focused on repairing the break, even at the cost of making some mistakes in the process.

On the left is double-stranded DNA that has been cut by the Cas9 protein, and on the right are three examples of this cut DNA being repaired by non-homologous end-joining. Closely compare the sequence of the three repaired DNA strands on the right to the original cut DNA sequence on the left, and circle any errors that were created in the repair process.



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What affect would these errors have on the resulting proteins created using this gene?

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Non-homologous end-joining works if your goal is to deliberately create errors in a gene that will stop it from functioning properly, often referred to as “knocking-out” a gene. But If your goal is to precisely edit or change a gene, you will want a second DNA repair system called ***Homology Directed Repair*** to fix the double- stranded cut in the DNA.

Homology Directed Repair (HDR)

If your goal is to edit a gene, you hope the cell will use the Homology Directed Repair system to repair the cut. HDR is more precise than NHEJ. HDR inserts a homologous piece of DNA into the cut site. This repair mechanism offers the possibility of replacing a defective gene with a functional version of the gene. But it does require that you provide the homologous DNA fragment, along with the Cas9 protein that will make the cut.

Homology directed repair (HDR) is a complicated molecular process and we will not attempt to explain it here. But know that HDR is similar to the “crossing-over” mechanism that results in the mixing of maternal and paternal chromosomes during meiosis. All of the proteins that participate in this process are present in your cells – just waiting until they are needed to repair damage to your DNA.

Minding Your Metaphors

The problem with the first generation of CRISPR-based approaches to genome editing is that we don’t have much control over which repair system goes to work on the cut that we create with Cas9. In that sense, the word “editing” is probably mis-used. An ***editor***, in the world of journalism, is someone who is very precise, and corrects small typos and other grammatical errors following some very well-established rules. With CRISPR, we make a sequence-specific cut (which is an amazing feat). . . but then just step back and hope for the best. The result is neither certain, nor even predictable. That is hardly editing.

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This hap-hazard repair event following the cut is OK if you are a researcher exploring this CRISPR technology in the lab, trying to swap in the gene encoding a green fluorescent protein into a defective globin gene in a culture dish containing a monolayer of several thousand mouse cells. In that case, you can easily detect and score the rare instances in which this happens because you can easily see those rare cells that begin to glow green. You can even selectively pick those green cells form the culture dish and clone them so that you now have a pure culture of green-glowing cells. But the challenge of editing a gene in a human person is much more challenging. The process must be proven to be highly efficient and safe (no off-target effects). And there is no opportunity to select only for those modified cells in which the editing goal was reached.

But all is not lost. We shouldn't despair too much. One lesson the science has taught us over and over again is "***never underestimate a clever molecular biologist***". Several research groups have already developed clever solutions to this daunting challenge of efficiently editing the human genome with CRISPR technology. In fact, two modifications of this CRISPR system have already been described that appear to make the prospect of human genome editing possible. To learn more, check out ***CRISPR Base Editing***, and ***CRISPR Prime Editing***.