What happens after the cut?

CRISPR Cas9 is a remarkable endonuclease that can be programmed with 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 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?

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. The first, and 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. This error-prone repair system often results in insertion and/or deletion events that inactivate the gene and its encoded protein. NHEJ is not a problem if your goal is to "knock-out" a gene. But it is a problem if you are trying to fix or "edit" the nucleotide sequence of a gene in a very specific way.

If your goal is to edit a gene, you hope that a second DNA repair system will try to fix the cut that Cas9 has introduced. This second repair system is more precise,… and is known as Homology Directed Repair (HDR). HDR inserts a homologous piece of DNA into the cut site and therefor 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 wit the Cas9 protein that will make the cut.

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, or even predictable. That is hardly editing.

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 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 tht science has taught us over and over again is,… don't underestimate a clever molecular biologist. I have no doubt but that several scary-smart research groups are right now developing clever solutions to this daunting challenge of efficiently editing the human genome with CRISPR technology. In fact, two clever modifications of this CRISPR system have already been described that appear to make the prospect of human genome editing possible. Stay tuned for a discussion of ***CRISPR Base Editing***, and ***CRISPR Prime Editing.*** Both of these new technologies are coming out of the David Liu lab at Harvard.