CRISPR: A Brimming Toolbox but No All-in-One

Commentary by Amélie Rezza, PhD 05/09/2017

If you have glanced at the science section of a newspaper in the past two years, you probably have heard of CRISPR, the most recent promising discovery in genome engineering. The Clustered Regularly Interspaced Short Palindromic Repeats system is indeed a powerful tool, courtesy of our little friends, bacteria. It is an adaptive immune system, the only one known in prokaryotes so far, that allows them to remember invading pathogens.[1] By integrating in their genome a short sequence of invading DNA, bacteria can later recognize the intruders if/when they come around again, and get rid of them.

Since its discovery, the CRISPR/Cas system has been modified and adapted for use in a plethora of eukaryotic cells with just one protein, a CRISPR enzyme that can cut DNA, and a single RNA molecule, targeting a specific sequence. This incredibly simple system can introduce double-strand breaks (DSB) wherever wanted in DNA, with single nucleotide precision. Some five years after its first use as a programmable system, every scientist now knows about it, and most have contemplated the ways in which it could boost their research.

From an elegant targeted DSB-inducing tool, CRISPR was transformed into a veritable brimming toolbox. In addition to being an efficient gene-editing tool, these days it is used to enhance crops, control transcription, induce epigenetic modifications,[2] and detect viruses (Zika and dengue so far) with high sensitivity and low cost,[3] to only list a few. One most interesting feature of this system is its application in screenings. This technique involves the use of libraries of single RNA molecules designed to hit numerous targets (sometimes more than 150,000).[4] It's been applied to systematically knock out more than 18,000 individual genes,[5,6] identify new fitness genes (genes involved in cell growth and proliferation),[4] and new cancer immunotherapy targets.[7]

Speaking of cancer, CRISPR discovery immediately triggered high hopes in therapeutics. Very few clinical trials have started so far (quite normal timing for a five-year-old technique, admittedly), but despite the overall enthusiasm, early concerns about the specificity of the system and its somewhat unpredictable off-targets are still not entirely lifted. Different studies have focused on identifying off-targets, including a recent, very controversial, quite alarmist report,[8] and many others describe enhancements of the system to lower these unwanted side effects.[9–11] These efforts might soon definitively resolve the off-target concern, but we're not quite there yet.

Even if/when the system has perfect specificity, there will still be obstacles to making CRISPR the perfect system for certain gene-editing applications, due to the very repair mechanism it triggers. Indeed, once the CRISPR enzyme has cut DNA, two different repair mechanisms can be induced: non-homologous end joining (NHEJ) results in small insertions or deletions (indels) at the DSB, whereas homology-directed repair (HDR) can integrate an exogenous donor DNA at the cut site. Quite annoyingly for gene-editing specialists, NHEJ is overwhelmingly predominant after CRISPR cuts, resulting in rather unpredictable indels, even when integrating donor DNA. These indels can come in handy as they can induce a frameshift, leading to a knockout, but they represent a major problem for precise gene editing, especially for point mutations or large knockins. As a result, the genome of any cell, clone, or animal obtained using CRISPR has to be carefully sequenced to determine the exact mutation that was introduced.

The unpredictable off-targets and indels might not be such an annoyance when working with cells, and it is the best, most efficient way to edit some species' genome (genetically modified rats are making a comeback), but when looking for a reliable, robust, precise way of making a transgenic mouse, classical homologous recombination (HR) might still be the safest way to go, especially for large knockins. Today, it is undeniable that CRISPR is a wonderful, useful system that has made gene editing easier, faster, and cheaper. But this is no all-in-one, and it still needs to be improved to qualify as the perfect, reliable and precise gene-editing tool.

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