The state of CRISPR research

April 9 2018, by Thomas Clements

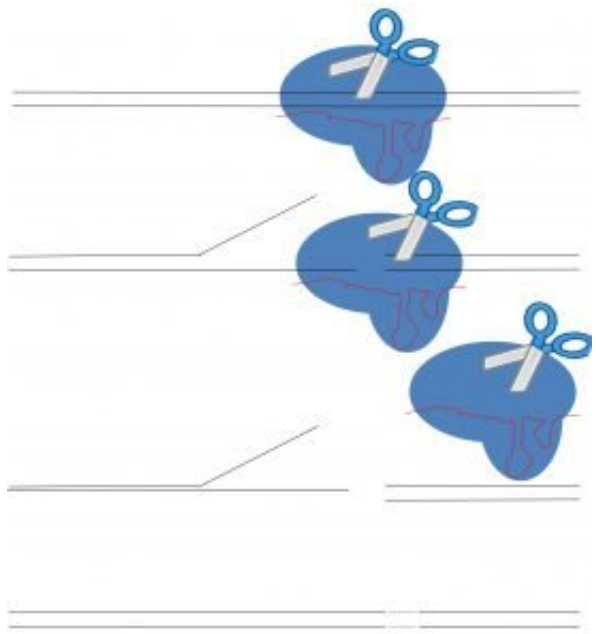

Figure 2: Cas9 mechanism. The Cas9 protein (blue) acts like a pair of scissors to cut DNA (black parallel lines) at specific location as directed by the sgRNA (red) to make a DSB. Once, the DNA is cut, deletions (shown by the dotted line) are often found at the cut site. Credit: T. Clements

CRISPR has been hailed as one of the most promising gene editing technologies and promises to revolutionize precision medicine and eradicate genetic diseases. However, the technique is not perfect and needs improvements. A new paper improves this system by creating a novel Cas9 fusion: ExoCas9. This fusion produces a higher rate of overall gene targeting and bias the spectrum of DNA lesions produced to

favor much longer deletions.

Genetic diseases have plagued society throughout human existence. These diseases result from mutations in an individuals' genome. The changes in the genome can be inherited or they can be acquired. Common genetic diseases include sickle cell anemia, Huntington's disease, Down's syndrome as well as predisposition to diabetes and even cancer. However, what if scientists could fix these mutations in an individuals' genome before they manifest in individuals? One the most up and coming, and hottest trends in biology is the use of the CRISPR-Cas9 system to edit DNA. CRISPR was first shown to modify DNA in 2012 (Gasiunas et al., 2012; Jinek et al., 2012) and since then, the number of publications about this technology has risen dramatically peaking in 2017 with over 3,000 individual publications. But how exactly does this technology work? How are scientists working to make it better? Will this technology be used to cure all genetic diseases in humans?

How do we edit DNA?

DNA is composed is four individual bases known as nucleotides (denoted A, C, T, and G). These nucleotides bind with each other and then wrap around to form a double helical structure.

In order to modify DNA, scientists often induce novel site-specific double-strand breaks (DSBs) in the DNA itself at specific locations. These DSBs have been commonly initiated through zinc-finger nucleases (ZFNs) (Beumer et al., 2008) and transcription activator-like effector nucleases (TALENs) (Cermak et al., 2011). Once a DSB break occurs, cells respond through two competing mechanisms: the dominant non-homologous end-joining (NHEJ) and homologous recombination (HR). Cells most often recruit repair machinery in NHEJ because it does not need a DNA template to be active as in HR. Nonetheless, the NHEJ

repair machinery often makes mistakes when repairing the DNA, which results in extra nucelotides (insertions) or missing nucleotides (deletions). As a result of these insertions or deletions, the genes no longer function normally.

However, both ZFNs and TALENs are cumbersome and expensive to design as well as assemble. This is because both tools require dimerization to cleave DNA and therefore two ZFNs or TALENs are required to target a specific site in the genome (Bitinaite et al., 1998). The clustered, regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated (Cas) endonuclease mechanism offers a simpler alternative these tools that is simpler in its design and also more amendable to high-throughput studies.

Figure 3: ExoCas9 mechanism. The ExoCas9 protein (blue) with the attached exonuclease (shown by the pink eraser) acts like a pair of scissors to cut DNA (black parallel lines) at specific location as directed by the sgRNA (red) to make a DSB. Once, the DNA is cut, the exonuclease erases the DNA further to make longer deletions (shown by the dotted line) at the cut site. Credit: T. Clements

How does CRISPR work?

The mechanism associated with CRISPR-Cas9 was originally discovered in bacteria and archaea where it served as adaptive immune system (Ishino et al., 1987). However, the type II CRISPR-Cas9 mechanism from S. pyogenes was adapted to modify DNA in living organism due to its simplicity. This system contains two components: the Cas9 protein, which acts like a pair of scissors in that it can cut DNA and single guide RNAs (sgRNAs), which act to recognize specific sequences on the DNA telling Cas9 where to cut (Chang et al., 2013; Hwang et al., 2013; Wei et al., 2013). The sgRNA first binds to the Cas9 protein and then leads it to the DNA of interest.

How we can make it better

Cas9 induced genome editing typically results in small insertions or deletions, typically on the order of