Not all genome editing tools are equal: CRISPR vs. TALEN

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January 27, 2021 -- In recent years, a number of novel genome editing tools have emerged, although not all of these tools are equal in terms of efficiency and accuracy, according to a new study published in *Nature Communications* on January 27. The study observed different DNA search proteins to identify the specific ways that they find target sequences in the genome.

Two types of programmable DNA search engines are CRISPR/CRISPR-Cas9 and transcription activator-like effector nuclease (TALEN). These proteins query genomic sequences searching for target-specific editing sites.

Cas9 can be programmed to find specific DNA sequences upstream of a protospacer-adjacent motif with the use of a single guide RNA that mediates target-site binding through DNA-RNA binding. Previous studies using dCas9 (nuclease-deficient Cas9) have shown a somewhat conflicting view of the Cas9 search mechanism, described as pure disassociation-reassociation events (3D diffusion) or sliding (1D diffusion) along DNA.

On the other hand, TALEN, which is made of customizable monomers that form a tandem array of 33/34 amino acids, can be assembled to recognize virtually any genetic sequence. In vitro studies show that TALEs (nuclease-free analogs of TALENs) utilize a unique rotationally decoupled "molecular zip-line" mechanism for a target-site search along DNA.

Genomic search of TALEN vs. Cas9 in open chromatin

To further describe the search mechanisms of dCas9 and TALE proteins, researchers from the University of Illinois Urbana-Champaign directly observed search behaviors of the proteins in different chromatin environments in vivo. The team used live-cell single-molecule fluorescence microscopy of designed TALE and dCas9 proteins targeting the cystic fibrosis transmembrane conductance regulator (CFTR) genomic loci and Alu retrotransposon elements.

To start, the researchers found that TALE and dCas9 proteins exhibited two types of search behaviors -- a "fast" diffusion and a "slow" diffusion -- and are capable of switching, suggesting that the proteins engage in global search as well as local search behavior.

Next, the team investigated the fundamental molecular differences of TALE and dCas9 target-search processes and how they affect editing outcomes. They found that dCas9 spends more time on nonspecific sites than TALE, and dCas9 spends more time undergoing local search compared to TALE.

The TALE target-search process is affected by genomic occlusions whereas dCas9 performs efficient genome search at the whole-nucleus level. This indicates that Cas9 can outperform TALENs in open chromatin.

Genomic search of TALEN vs. Cas9 in compact chromatin

In live mammalian cells, the genome is arranged in heterochromatin structures (tightly condensed inactive DNA). So, to elucidate search dynamics in heterochromatin environments, the researchers imaged TALE and dCas9 search dynamics in Alu repetitive retrotransposons, centromeric structures, and a compact genomic locus marked by H3K9 trimethylation epigenetic modifications.

Using a fluorescent tag, the team was able to track single-protein molecules in heterochromatin. Search dynamics indicated that TALE could maneuver a tight heterochromatin environment more efficiently than dCas9.

To assess the functional differences in search behavior, the team next constructed a series of TALENs and Cas9-gRNA variants for their ability to edit sequences in highly repressed heterochromatin loci. In 11 out of 12 loci, TALENs showed similar or higher editing activity in heterochromatin compared to Cas9. In some cases, the researchers observed a fivefold increase in editing efficiency for TALEN compared to Cas9 in heterochromatin regions.

However, at four euchromatin sites (compact chromatin under active transcription), Cas9 demonstrated either similar or greater editing activity. The authors suggested that Cas9 is more efficient in cutting at euchromatin sites due to its greater ability to query binding sites in a relatively unhindered environment.

The study adds to the evidence that a broader selection of genome editing tools is needed to target all parts of the genome, said Huimin Zhao, PhD, a professor of chemical and biomolecular engineering at the University of Illinois Urbana-Champaign, who led the new research.

"We found that CRISPR works better in the less-tightly wound regions of the genome, but TALEN can access those genes in the heterochromatin region better than CRISPR," said Zhao in a statement. "We also saw that TALEN can have higher editing efficiency than CRISPR. It can cut the DNA and then make changes more efficiently than CRISPR."

The findings will lead to improved approaches for targeting various parts of the genome, Zhao said.

"Either we can use TALEN for certain applications, or we could try to make CRISPR work better in the heterochromatin," he said.