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An alternative CRISPR-cas nuclease for **precise genome editing**

Dr Michael Krohn, head of R&D at **BRAIN Biotech**, explains the importance of genome editing in living organisms for the bioeconomy and describes the development of the company's novel Cas nuclease

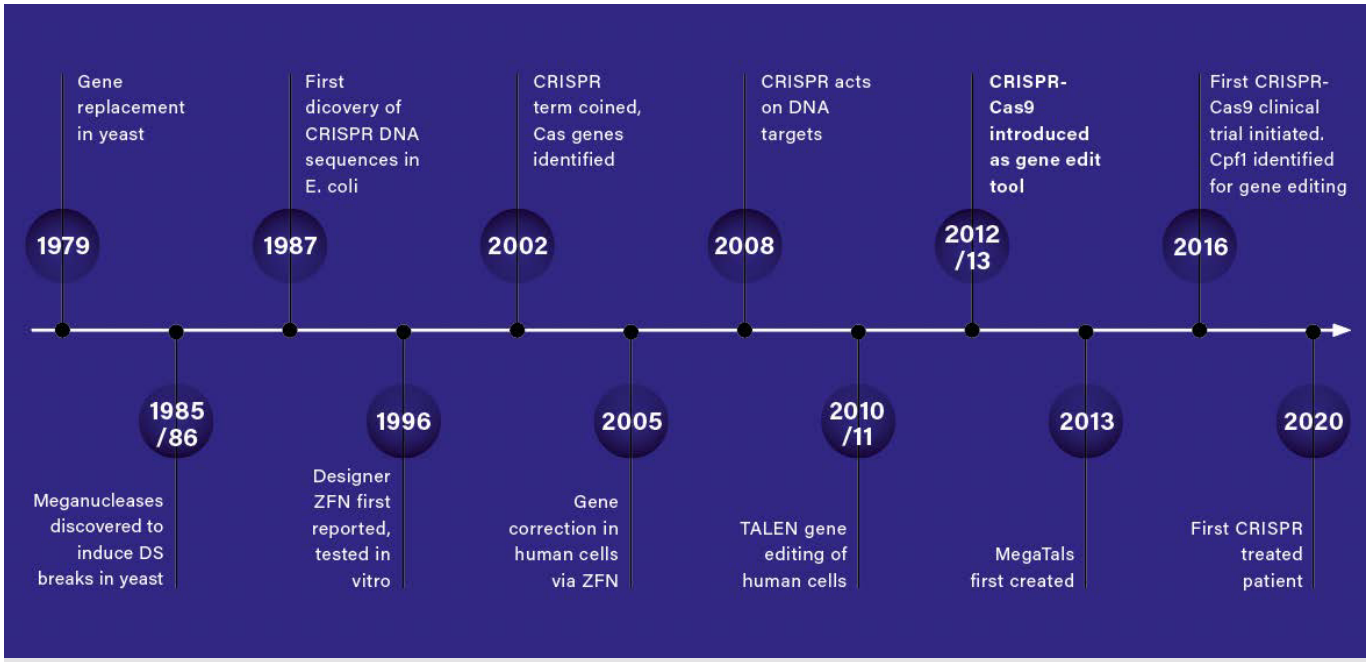

Figure 1 - Chronological development and type of targeted genome modifications Note: MegaTals = fusions of meganucleases and Talens. © BRAIN Biotech up the side

The goal of modifying genes and thus the properties of organisms has been pursued by science since more and more knowledge of molecular genetics was gained with the invention of new technologies.

The idea of optimising crops or curing hereditary diseases drove the search for gene modification methods. After all, waiting for organisms to adapt to certain environmental conditions as part of evolutionary development is not an option, especially against the backdrop of climate change.

From non-specific to specific

In classical gene modification approaches, which are still used today as non-GMO methods in agriculture, organisms are treated in laboratories by irradiation or with mutagenic chemicals to induce changes in the genome. This procedure is not really specific.

Moreover, until recently it was hardly possible to analyse how the genome had changed as a result of the treatment. Only external appearance characteristics (phenotype) allowed conclusions to be drawn about 'successful' gene modification. Additional, unwanted genome changes remained undetected or persisted as silent accompaniers.

Genome editing is a newer method of molecular biology to edit genomic DNA precisely at predetermined locations. The technique facilitates a hitherto unprecedented, targeted and precise insertion, deletion or modification of the genome of living organisms and therefore offers the opportunity to optimise non-specific modifications with specific or targeted modifications.

Today, modern research in molecular biology uses different tools for genome editing. What they all have in common is that they work on the basis of nuclease enzymes that specifically recognise a gene locus and do so by interacting DNA-binding domains of the nuclease(s) with a sequence of bases in a predetermined genome section that is as unique as possible.

This class of nucleases, which usually induce cleavage of bound DNA, includes zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) or meganucleases (Figure 1). In the early 21st century, clustered, regularly interspaced, short palindromic repeat (CRISPR)-Cas systems were discovered and with them systems that function differently. In CRISPR systems, a specific RNA mediates the recognition and targeting of DNA.

With the discovery of *CRISPR-Cas systems (Cas9, Cas12 or others) and their ability to modify DNA precisely, a tool had been discovered that, by following nature, revolutionised the biotechnologically-induced evolutionary process of living organisms. With the emerging technology, which was first applied in 2012, the selection process could not only be carried out in a targeted and precise manner, it could also be accelerated enormously.

Biotechnology can therefore now selectively insert, remove or modify individual DNA segments in living organisms in a relatively simple process. Some people are already talking about so-called garage technology for programming microorganisms that can be used industrially. An ingenious technology – were it not for the dispute over patents, which makes the current legal situation for commercial use complex and uncertain and also demands high licensing fees from companies for using the technology.

The uncertain legal situation and the licensing agreements for the commercial use of CRISPR technology, which are financially unsustainable for many companies, have motivated us to develop our own variant of the CRISPR-Cas scissors with the BRAIN-Engineered-Cas (BEC) nuclease. This enables us, for example, to optimise the metabolic performance of microbial production strains within a short time.

In view of this licensing situation, we turned to our technological expertise and set ourselves the goal of developing our own nuclease. We used metagenome sequencing and protein engineering techniques to identify a Class 2 CRISPR-associated nuclease that has low sequence homology to other CRISPR nucleases and targets DNA with a unique molecular mechanism. We have filed a patent to protect the nuclease DNA sequence and expect to be able to use this system freely in the future.

'We CRISPR for you'

We not only use our validated CRISPR-associated BEC nuclease for targeted gene editing in our own projects, but also make it available for customer projects. Organisms in which BEC targets DNA include bacteria, fungi and yeasts.

Accordingly, the technology is being used in these organisms for collaborative projects and is also being progressively validated for industrial use. Activity in plants has also been demonstrated, but validation is pending. Genome editing tests for other applications, such as mammalian cell lines, are currently underway.

But what exactly does the use of this nuclease mean for users? The technology is an enabling technology and allows the user to precisely optimise microorganisms according to his own specifications.

Once established in the target organism, genome editing is not only precise, but also efficient and fast. It is often superior to conventional technologies in terms of time to market: the development of, for example, highly efficient producer strains can be significantly accelerated.

Further potential applications of BEC technology lie in the food industry. In some countries, genome editing is already applied in food manufacturing processes, including precision fermentations to obtain alternative proteins from microbial or plant sources or optimisation of microbial strains to produce fermented beverages.

In order to drive sustainability in companies, the chemical industry can switch part of the chemical processes to biological processes. Here, for example, BEC nuclease can modify microbial strains to use a specific organic substrate as a carbon source and to engage in synthesis pathways in the course of which the microorganisms produce bio-based building blocks and platform chemicals. Converting

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waste streams into valuable products by biotechnological means can therefore lead to new and sustainable value chains.

In the agro industry, the optimisation of plants in terms of their resistance to climate change, pest resistance, vertical farming, etc. is conceivable. Positive prospects should then include, for example, lower water consumption, reduced pesticide inputs into nature or more efficient land use for the production of crops.

Contribution to the bioeconomy

Developing new technologies is at the heart of BRAIN's business. However, we do not just optimise microorganisms. We also identify and develop optimised enzymes and biocatalysts that meet complex process and application requirements, such as enzymes for the production of food, beverages, lubricants or starch.

If a particular enzyme is not found in the BRAIN Group's enzyme portfolio, our metagenome libraries are the basis for our sequence- or activity-based search for new enzymes. Although we see ourselves as biotech specialists, we cover the entire value chain, from conception and development to process development or production. In this way, we contribute to the development of the urgently needed bioeconomy. ●

Figure 2 - Culture plate with microbial cell colonies at BRAIN lab. *Successful genome editing seen in the form of red staining*

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