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THÈME

La technologie CRISPR/Cas9

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Dédicace

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Special section for my chingudeul: You are the stars of my universe…

Liste des abréviations

CRISPR: Clustered regularly interspaced palindromic repeats

Cas: CRISPR associated protein

DNA: Deoxyribonucleic acide

RNA: Ribonucleic acid

PAM: protospacer adjacent motif

DS: double strand

DMD : Duchenne Muscular Dystrophy

FA : Fanconi Anemia

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Introduction

Introduction

In the early twentieth century, physicians and chemists unlocked secrets of the atom that changed the world forever. But life remained a profound mystery. Among life's deepest secrets was inheritance. Everyone knew that traits like shape of a peapod or the color of eyes and hair were passed on from generation to generation. But no one knew how such information was stored or transmitted. Scientists were convinced that were had to be a biological molecule at the heart of the process. And that molecule had to have some special qualities. The challenge of solving this mysterious "secret of life" and, furthermore finding a way to edit it and change it which is now known as genetic engineering was taken up (Doudna, 2015).

Genetic editing before DNA discovery:

Genetic engineering is the process of changing the genetic makeup of an organism be it an animal, plant or micro organism, using certain biotechnology techniques that have only existed since the 1970s. However, human directed genetic manipulation was occurring much earlier, beginning with breeding animals and crops with beneficial traits through: selective breeding, inbreeding and hybridization

- Selective breeding is a technique that involves selecting parents that have characteristics of interest so that the resulting offspring have desirable traits.
- Inbreeding is the production of offspring from the breeding of individuals or organisms that are closely related genetically. Inbreeding is useful in the retention of desirable characteristics or the elimination of undesirable ones.
- Hybridization which is another breeding technique that involves crossing dissimilar individuals to bring together the best traits of both organisms and to create a hybrid.

All these techniques helped people in their daily life so they started using it so often, and it became frequent and got transmitted from generation to generation, however, they never understood how it works or how these traits are expressed, and the secrets of humanity genes kept in the unknown... until the discovery of DNA (Ross, 2019).

The discovery of DNA in 1970 and the beginning of genetic editing:

Several researchers over many year discovered that in the nucleus of our cells there was a substance called DNA which contained all our genetic information Like a recipe book it holds the instructions for making all the proteins in our bodies. And DNA stands for deoxyribonucleic acid, it's made up of molecules called nucleotides attached together to make a polynucleotide which forms each strand of the double helix.

Each nucleotide contains 3 principle ingredients: a 5 carbon sugar molecule (deoxyribose), a phosphate group, and one of the four nitrogen bases (adenine, guanine, thymine, and cytosine). The order of these nitrogen bases is what forms genes. The 2 strands of the DNA run in opposite directions, and the bases on each strand are linked together, adenine with thymine while cytosine links to guanine. . These 2 chains spiral to create the helix. And to fit inside the cell, the DNA coils to form chromosomes (DNA: Definition, Structure & Discovery, 2017).

As soon as this revolutionary discovery happened scientists tried to manipulate it using different techniques like radiation and chemicals which cause random mutations in the DNA (1960s). In the 70s scientists discovered new technologies for sequencing, copying and manipulating DNA and they started using them on many different cells for research and medical reasons (Bagley, 2013).

These Technologies for making and manipulating DNA were really promising and have enabled advances in biology ever since the discovery of the DNA double helix.

But until recently, it's been very hard to rapidly obtain the results of their experiments, because these techniques were taking lot of time and effort and sometimes they were inefficient. In addition to the fact that introducing site-specific modifications in the genomes of cells and organisms remained elusive. Early approaches relied on the principle of site-specific recognition of DNA. More recently, the site-directed zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs) using the principles of DNA protein recognition were developed. However, difficulties of protein design, synthesis, and validation remained a barrier to widespread adoption of these engineered nucleases for routine use (Doudna & Charpentier, 2014).

But, all this changed when a new technology called “CRISPR” appeared... and it was like someone has pressed fast-forward on the gene-editing field: A simple tool that scientists can wield to snip and edit DNA is speeding the pace of advancements that could lead to treating and preventing diseases (Doudna & Charpentier, 2014). CRISPR stands for "clusters of regularly interspaced short palindromic repeats."

Timeline of gene editing (Illman, 2017)**:**

1856 – 1863	Father of genetics	Gregor Mendel
1869	Identifying DNA	Friedrich Miescher
1953	**DISCOVERY OF DNA STRUCTURE**	Francis Crick and James Watson
1961	Cracking the DNA code	Marshall Nirenberg
1977	DNA sequencing	Frederick Sanger
1983	Copying DNA	Kary Mullis
2002	CRISPR	Dutch scientists first coined the term CRISPR
2003	Completion of the human genome project	
2005	Discovery of Cas9 protein	
2012	CRISPR-Cas9 Editing tool	Jennifer Doudna and Emmanuelle Charpentier

Bibliographie

Chapitre I
What is CRISPR?

Chapter 1 : What is CRIPR?

Chapter 2

1.1.Definition

If you're asking, "what's CRISPR-Cas9?" the short answer is that the CRISPR technology is a revolutionary new class of molecular tools that scientists can use to make changes in any kind of genetic material, it involves the precise targeting, cutting and pasting of the DNA in cells. Crispr systems are the simplest yet powerful methods scientists have ever had to alter every DNA sequence on Earth, and humans' are included.

The long answer is that CRISPR stands for Clustered Regularly Interspaced Palindromic Repeats. CRISPRs are repeating sequences found in the genetic code of bacteria. They are interspersed with 'spacers' — unique stretches of DNA that the bacteria grab from invading viruses, creating a genetic record of their malicious encounters.
Crispr systems consist of a scissor-like protein called Cas9 and a genetic GPS guide "The guide RNA= gRNA". Such systems inspired by nature and engineered by researchers naturally evolved across the bacterial kingdom as a way to foil attacks by viruses and other foreign bodies. But researchers reoriented and repurposed that primordial immune system to precisely manipulate genomes (Doudna, 2015).

1.2.How it first discovered?

Years ago, sequences of clustered, regularly interspaced short palindromic repeats (CRISPRs) were found disseminated in the genomes of numerous bacteria. In 1987, the first description of a CRISPR array was made by researchers who found repeats of multiple base pairs (bp) that were interspersed by bp non-repeating spacer sequences in Escherichia coli. In 1995, similar CRISPR arrays were found in Mycobacterium tuberculosis, Haloferax mediterranei, and other bacteria and archaea. Several hypotheses for the function of CRISPRs have been proposed. But their function as a safeguard and a defense system against viruses was not highlighted until 2007, when some research groups reported that the spacer sequences often contained parts of phage-derived DNA or plasmids, and with these extrachromosal agents they proposed that CRIPR is using them to mediate immunity against infection. Other researchers also reported on a negative correlation between the number of CRISPR spacers in the genome of bacteria and their sensitivity to phage infection. Few years after it, some scientists confirmed this hypothesis experimentally by showing that after a phage invading, new spacers were acquired that conferred resistance against the phage (Rotem Sorek, 2008).

In 2012, and through a basic research project on how bacteria fight viral infections, Jennifer Doudna and her colleague Emmanuelle Charpentier invented this new technology for editing genome using the CRISPR-Cas9 (CRISPR Associated Proteins 9) system (Doudna, 2015).

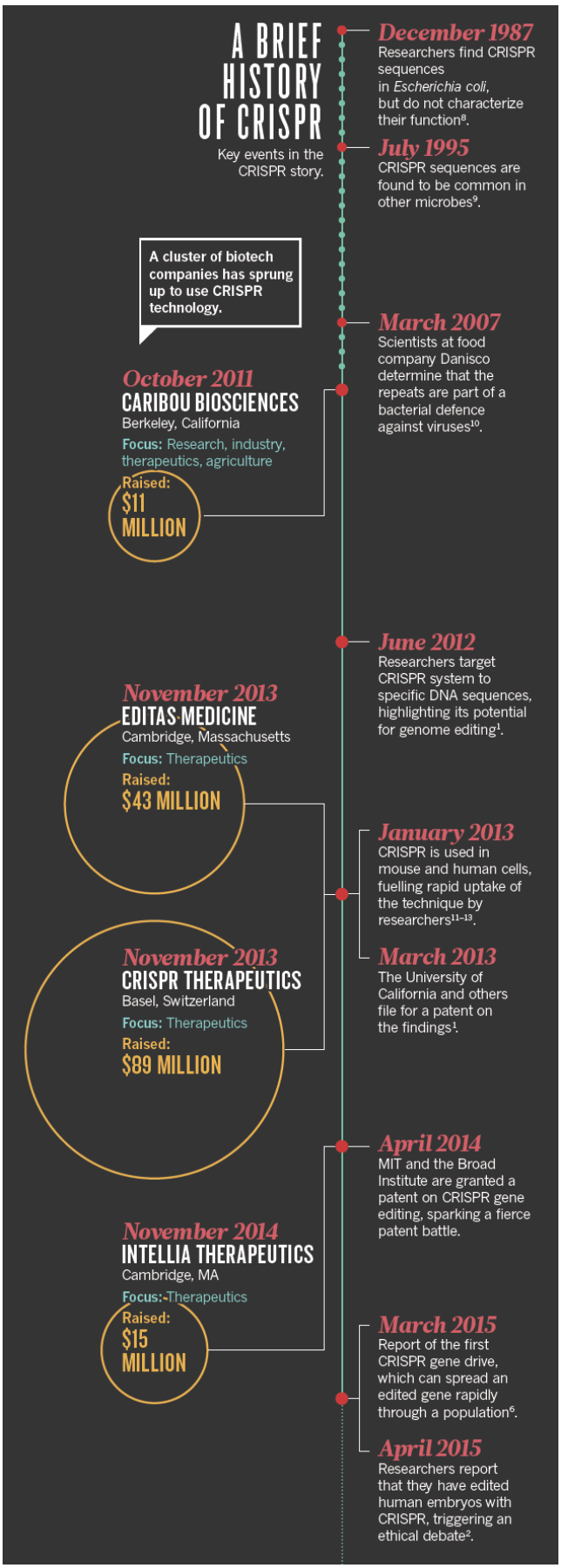


Figure 1: a Brief history of CRISPR (Ledford, 2015)

1.3.How it works?

- **The CRISPR lexicon**

CRISPR loci: are found solely in the genome of archea and bacteria, they contain the CRISPR arrays including:

The leader sequence: a sequence that contains hundreds of nucleotides and where the transcription of the array CRISPR starts. Researchers found that a CRISPR loci lacking the leader sequence is unable to incorporate new spacers and to excute the CRISPR-Expression and interference (Sinan Al-Attar, 2011).

The associated Cas genes.

CRISPR array: sequences of clustered (cluster means groups), regularly interspaced palindromic (that has the property of reading the same forwards as it does backwards) repeats found in the genome of many bacteria and almost all archea.

These arrays are composed of:

Direct repeats "the short palindromic repeats" that are separated by similarily sized non-repetitive spacers "protospacers" which originate from either phages or plasmids and comprise the prokaryotic "immunological memory" (Rotem Sorek, 2008).

CRISPR associated proteins (Cas): enzymes that has a role in the mediated CRISPR resistance, there are nucleases, helicases, integrases and polymerases. These activities have a role in addition and suppression of new spacers, processing of the CRISPR-Transcripts and mediation of other CRISPR-defense processes (Sinan Al-Attar, 2011). The Cas9 is an endonuclease found in CRISPR type II and guided by two RNAs (Jennifer A. Doudna, 2012).

CRISPR RNA (crRNA): generated from the CRISPR loci. Their function is to guide Cas proteins to silence invading nucleic acids.

The CRISPR array is transcribed to make the precursor-CRISPR-RNA (pre-crRNA). Under the co-processing of transcript-activating crRNA (tracrRNA) and RNas III the maturation of the pre-crRNA happens and it becomes a crRNA. The dual tracrRNA crRNA with the Cas9 form then a complex to cleave site-specifically target DNA (Chylinski *& al*, 2013). Now, the dual tracrRNA:crRNA was engineered as a single guide RNA (sgRNA).

Protospacer Adjacent Motif (PAM): a few short 2-5 base pairs (bp) sequences found adjacent to one end of the protospacers, linked to the excision of protospacers and their insertion into CRISPR loci (Shah *& al*, 2013).

Plasmids carrying out a protospacer sequence but no PAM are resistant to CRISPR-Cas9 cleavage (Gasiunas *& al*, 2012)

- **Functionality of CRISPR-Cas9**

Bacteria have to deal with viruses in their environment**.** A virus infection is a ticking-time bomb, a bacterium have only a few minutes to diffuse it says Jennifer Doudna. Bacteria have in their cells CRISPR as an adaptive immune system that allows detecting viral DNA, store a record of it and destroy it upon re-exposure (Doudna, 2015).

The CRISPR mediate resistance is a multistage process that functions in a three distinct steps that provide DNA-encoded, RNA-mediated and sequence-specific targeting of exogenous nucleic acids (Barrangou, 2015).

1. **Adaptation**

When virus infect a cell and inject their DNA, pieces of this viral DNA are sampled from the invasive by the CRISPR system. Specialized Cas proteins insert it into the "CRISPR loci" and they are acquired as novel "spacers", They serve as a bank of memories, which enables bacteria to recognize the viruses and fight off future attacks in a sense, the spacers in CRISPR are an account of the bacteria's battlefield wins (Staedter, 2017). The spacers' acquisition is the first step of immunization (Barrangou, 2015). These bits of viral DNA (spacers) will serve as a record of infection over time to the viruses they have been exposed to. Moreover, these bits are passed on to the cell's progeny (offspring), resulting in the protection from viruses not only in one generation as Blake Wiedenheft referred to the CRISPR loci as a genetic vaccination card (Doudna, 2015).

2. **Expression**

After inserting the spacers, the CRISPR array is transcribed and processed to make precursor-CRISPR RNAs (precrRNA) an exact replicate of the viral DNA. The maturation of the precrRNA to the crRNA requires the presence of a trans-activating crRNA (tracrRNA) co-processed by RNAS III. The final product is a crRNA with the size of a spacer-repeat unit (Sinan Al-Attar, 2011).

3. **Interference**

Through sequence homology, the dual tracrRNA:crRNA guides the Cas9 endonuclease. The recognition of invaders nucleic acids then is done by complimentarily to the crRNA. The guide RNAs direct the Cas for specific targeting, cleavage and degradation of complementary nucleic acids (Barrangou, 2015). It searches the DNA in the cell, and when matching sites are found, the complex (tracrRNA:crRNA:Cas9) associates with the DNA and allows Cas the cleaver to cut up the viral DNA by making a double-stranded (ds) break into the DNA helix and stopping the virus from replicating (Doudna, 2015).

On a repeat encounter with a virus, a bacterium can produce a stretch of RNA that matches the viral sequence, using the material in its spacer archive (Doudna, 2015).

Finally, There is a built-in safety mechanism, which ensures that Cas9 doesn't just cut anywhere in a genome. Short DNA sequences known as PAMs "protospacer adjacent motifs" serve as tags and sit adjacent to the target DNA sequence (Sander & Joung, 2014). They are essential for cleavage of the target DNA during the interference stage. If the Cas9 complex doesn't see a PAM next to its target DNA sequence, it won't cut. It is a strategy CRISPR uses to distinguish self from non-self sequences and that is the reason why the Cas9 doesn't ever attack the CRISPR region in bacteria. Known Cas9 proteins will target only ds DNA sequences followed by a 3′ PAM sequence specific to the Cas9 of interest. Cas9 rapidly dissociates from DNA that does not contain the appropriate PAM sequence, whereas it binds for a longer duration at sites containing a PAM sequence, with the dwell time depending on the degree of complementarity between the guide RNA and the adjacent DNA. Once Cas9 has found a target site with the appropriate PAM, it triggers DNA unwinding from the PAM-proximal end to the PAM-distal end of the target site (Chen & Doudna, 2017).

Scientists tried to understand the activity of cas9 and how they could harness its function as a genetic engineering technology to offer opportunities to do things that really haven’t been possible in the past. And they found that it can programmed to recognize particular DNA sequences and make a break at that site, this activity is now used in genome engineering. That allowed scientists to make a very precise change in the DNA at the site where the break was introduced (Doudna, 2015).

And the reason why the CRISPR system can be used for genome engineering is that cells have the ability to detect broken DNA and repair it (Eastman & Barry, 1992), so when a plant or an animal cell detects a ds break in its DNA, it fixes it either by pasting together the ends of the broken DNA or it can repair the break by integrating a new piece of DNA at the site of the cut. So CRISPR is the way to introduce ds breaks into DNA at precise places and by that we can trigger cells to repair those breaks either by disruption or incorporation of new genetic information (Doudna, 2015).

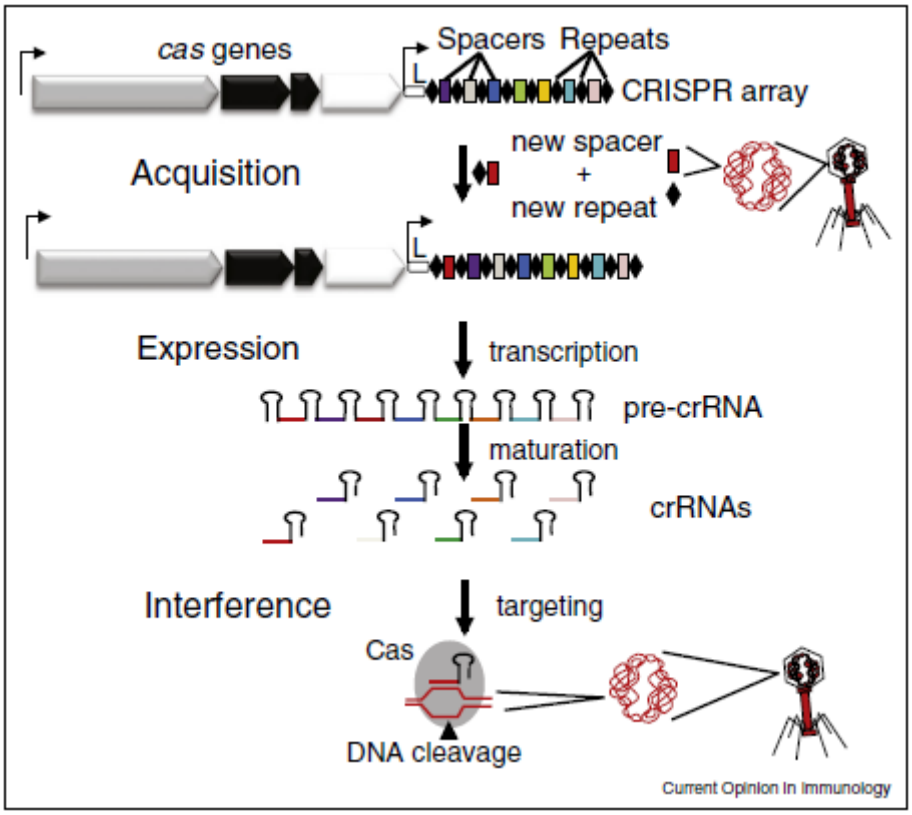


Figure 2: CRISPR–Cas immune systems CRISPR-encoded immunization and interference (Barrangou, 2015)

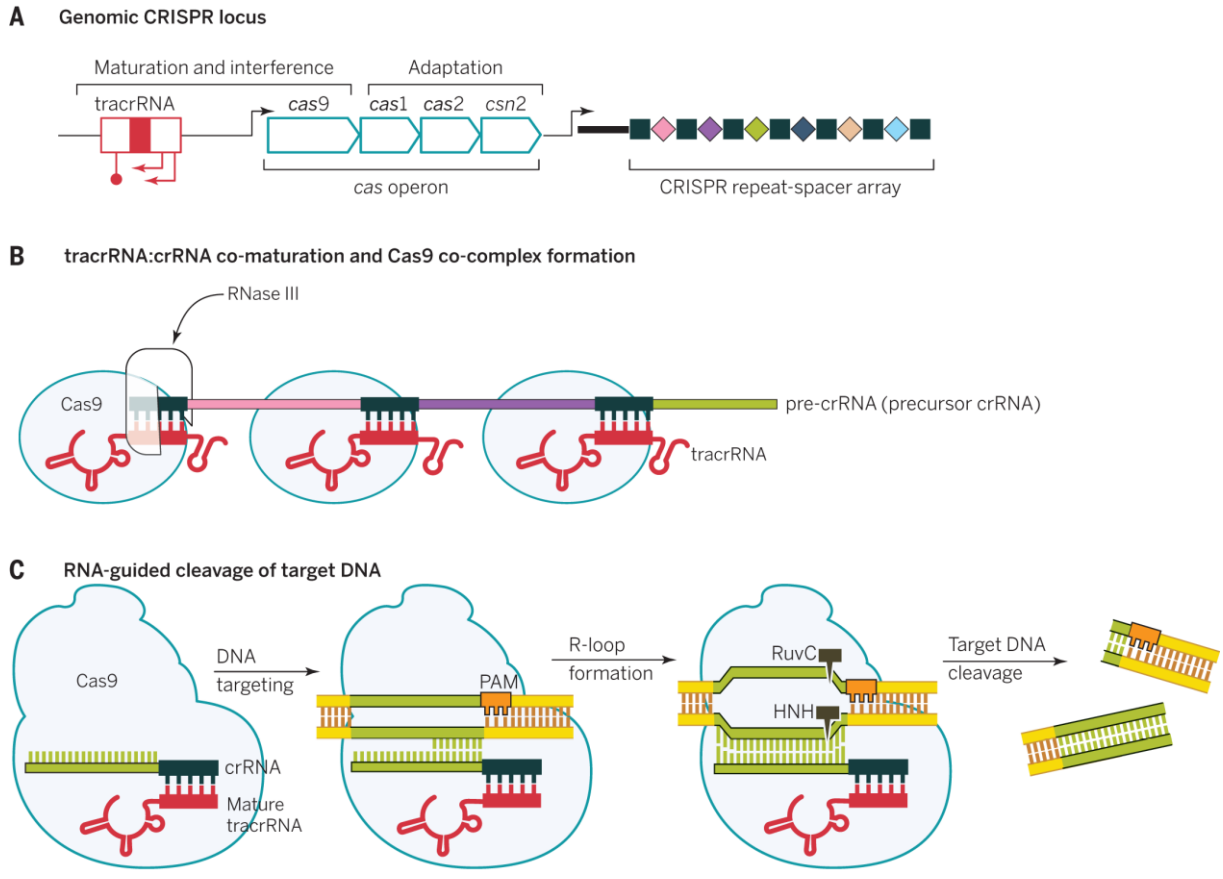


Figure 3: Biology of the type II-A CRISPR-Cas system from S. pyogenes (Doudna & Charpentier, 2014)

- **Genome engineering using CRISPR**

The CRISPR/Cas technology has emerged as the most popular tool for the precise alterations of the genomes of diverse species and different organism models. CRISPR/Cas9 system has taken the world of genome editing by storm in recent years, making it one of the hottest technology breakthroughs. Its popularity as a tool for altering genomes is due to the ability of Cas9 protein to cause double-stranded (ds) breaks in DNA after binding with short guide RNA molecules, which can be produced with dramatically less effort and expense than required for production of transcription activator like effector nucleases **(**TALEN) and zinc-finger nucleases (ZFN). This system has been exploited in many species from prokaryotes to higher animals including human cells as evidenced by the literature showing increasing sophistication and ease of CRISPR/Cas9 as well as increasing species variety where it is applicable. This technology is poised to solve several complex molecular biology problems faced in life science research. The invention of single guide RNA (sgRNA) by fusing crRNA and tracrRNA was an important breakthrough in this field because it simplified the task of programming Cas9 to create breaks at specific DNA sites *in vitro.* Following this advance, this technology has been adopted for genome engineering in cells and different model systems

The first step towards gene alteration is precise generation of single- or double-stranded breaks (SSB or DSB) in the genome. Cas9/sgRNA complexes can generate accurate breaks in the genomes of bacteria, yeast, plants and animals. CRISPR-Cas9 is the latest inclusion in the toolbox of genome editing that already contains (ZFNs) and (TALENs). The CRISPR/Cas9 system is currently the most desirable tool for genome engineering for several reasons. Cas9 is programmed by readily engineered sgRNAs. The ease of CRISPR/Cas9 use has opened new prospects for studying functional genomics of diverse organisms in a more precise manner with less effort when compared to other techniques like TALENs and ZFN the variety of applications is growing at a rapid pace to include different types of knock-outs at single-gene or genome-wide scale, knockings, activating and inhibitory Cas9 versions, visualization tools and even biochemical (Ceasar *& al*, 2016).

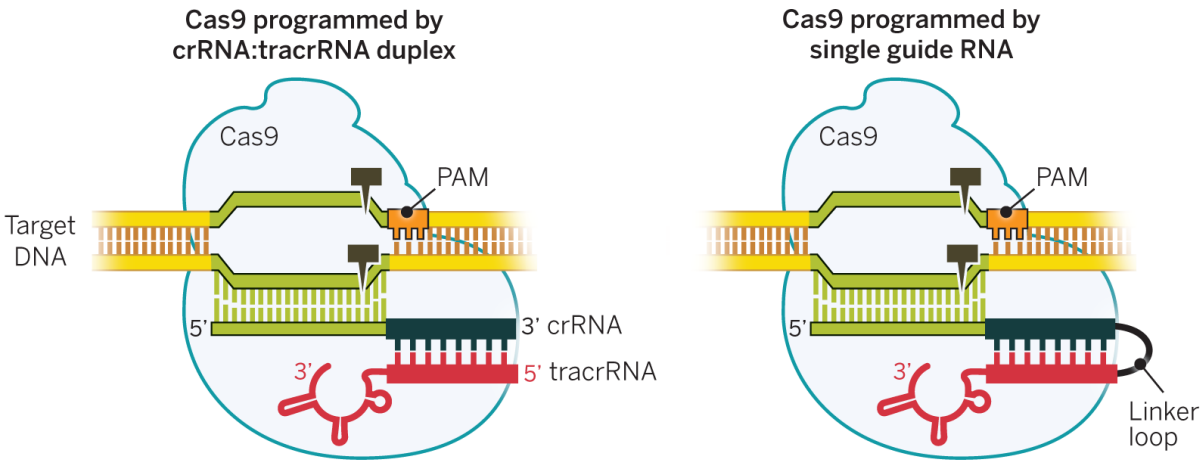


Figure 4: Evolution and structure of S. pyogenes Cas9 (Doudna & Charpentier, 2014)

1. Generating a knockout using CRISPR

Knockout: Permanently disrupt gene function in a particular cell type or organism without a specific preferred mutation.

Cas nucleases enable efficient and precise genetic modifications by inducing targeted DNA double-strand breaks (DSBs) that stimulate the cellular DNA repair mechanisms, including error-prone non homologous end joining (NHEJ) and homology-directed repair (HDR). The Cas9:gRNA complex binds to the target DNA to cleave the target DNA from 3' to 5'. Then the Cas9 undergoes a conformational change that positions the nuclease domains (RuvC and HNH) to cleave opposite strands of the target DNA that results in a ds break. This break in the DNA incites repair pathways, the efficient but error prone NHEJ or the less efficient but high-fidelity HDR. The NHEJ is the most active repair mechanism that causes small nucleotide insertions/deletions at the ds break sites. Diverse arrays of mutations appear that causing amino acid insertion/deletion/frameshift mutation that leads to premature stop codons of the target genes. The ideal result is a loss-of-function mutation within the targeted gene resulting in the knockout of the phenotype (Chen *& al*, 2015).

2. Enhancing specificity with Nickases

Cas 9 generates ds breaks through the combined activity of the two nuclease domains RuvC and HNH. Cas nickase is another advantage of CRISPR that can be converted to a nickase that creates single-stranded (ss) breaks by retaining only one nuclease domain and generating a DNA nick rather than a ds break. Either of the two endonuclease domains of Cas9, HNH and RuvC can be mutated to form nickases. That transforms the Cas9 complex into a strand-specific nicking endonuclease **(**Gasiunas *& al*, 2012**).**

3. Activation or repression of target genes using CRISPR

Repress (knockdown): reduce expression of particular gene(s) without permanently modifying the genome.

Activate (CRISPRa): increase expression of an endogenous gene(s) without permanently modifying the genome.

RuvC and HNH domains can be rendered inactive by point mutations, resulting in a nuclease dead Cas9 (dCas9 lacking endonuclease activity) molecule that cannot cleave target DNA. The dCas9 molecule retains the activity to bind to target DNA based on the gRNA targeting sequence. This system is called CRISPR interference (CRISPRi). Early experiments demonstrated that targeting dCas9 to transcription start sites was sufficient to repress transcription by blocking initiation. dCas can also be tagged with transcriptional repressors or activators (Gilbert *& al*, 2013), and targeting these dCas9 fusion proteins to the promoter region (the section of DNA that controls the initiation of RNA transcription) results in rebust transcriptional repression or activation (it can upregulate endogenous expression in human cells (Cheng *& al*, 2013), of downstream target genes (towards the 3' end). The simplest dCas9-based activators and repressors, consist of dCas9 fused directly to a single transcriptional activator or repressor. Importantly, unlike the genome modifications induced by Cas9 or Cas9 nickase, dCas9-mediated gene activation or repression is reversible, since it does not permanently modify the genomic DNA (Qi *& al*, 2013).

4. CRISPR base editing without double-strand breaks

Base editing is a different approach to genome editing that enables the direct, programmable, targeted point mutations without inducing ds breaks by the conversion of a C:G base pair to T:A base pair.

CRISPR base editors fuse Cas9 nickase or dCas9 to a cytidine déaminase. Base editors can convert cytidine to uridine within a small single-stranded DNA bubble at a guide RNA-specified locus near the PAM site. Base excision repair (BER) is the cell's primary response to G:U mismatches, creating a C->T change (or G->A on the opposite strand) conversion in mammalian and plant cells. In addition, new base editors have been engineered to convert adenosine to inosine, which is treated like guanosine by the cell, creating an efficient and permanent A->G (or T->C) change on the opposite strand (Rees *& al*; Komor *& al*, 2017).

5. Epigenetic modification using CRISPR

Epigenetics have several meanings. To Conrad Waddington, it was the study of epigenesis: that is, how genotypes give rise to heritable phenotypes changes without altering the DNA sequence (Waddington, 1957).

Cas enzymes can be fused to epigenetic modifiers to create programmable epigenome engineering tools that could be be used to precisely control cell phenotype or the relationship between the epigenome and transcriptional control. Like CRISPR activators and repressors, these tools alter gene epression without inducing a ds break. However, they are much more specific for particular chromatin and DNA modifications, allowing researchers to isolate the effects of a single epigenetic mark. Another potential advantage of CRISPR epigenetic tools is their persistence and inheritance. CRISPR activators and repressors are thought to be reversible once the effector is inactivated/ removed from the system. In contrast, epigenetic modifiers may be more frequently inherited by daughter cells (Hilton *& al*, 2015).

6. Visualize genomic loci using fluorophores

The fluorescence labeling of endogenous genomic DNA by CRISPR using a dCas9 has greatly simplified study of the spatial organization of the genome in live cells with numerous advantages over other techniques including the simplicity of programming to target wide arrays of deferent genomic sequences even detecting multiple genomic loci. This method offers a unique detecting of the chromatin dynamics in living cells (Ma *& al*, 2015)

7. . Multiplex genome engineering with CRISPR

CRISPR cas9 allows for simultaneous targeting of multiple genomic loci. This multiplexing feature uses multiple guide RNA sequences that can be encoded into a single CRISPR array to enable simultaneous editing of several sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology

the ability to carry out multiplex genome editing in mammalian cells enables powerful applications across basic science, biotechnology, and medicine (Cong *& al*, 2013).

8. RNA targeting

In some bacteria, type VI CRISPR systems recognize single-stranded RNA (ssRNA) rather than dsDNA. RNA-guided RNA-targeting type VI CRISPR is capable of highly efficient and specific RNA knockdown and degradation in mammalien cells.

Type VI CRISPR-Cas systems contain the programmable single-effector RNA-guided ribonuclease Cas13. Similar to Cas9, Cas13 can be converted to and RNA-binding protein through mutation of its catalytic domain (Cox *& al*, 2017).

9. Gene Drive

Gene drives are a particularly powerful application of CRISPR technology. They are genetic elements that insert themselves into target sites lacking that element, convert heterozygous alleles to homozygous alleles within an organism by allowing CRISPR on one chromosome to copy itself to its partner in every generation,. In organisms that support sexual reproduction, gene drives enable non-Mendelian inheritance of alleles that can spread throughout a population. Usually, a genetic change in one organism takes a long time to spread through a population. That is because a mutation carried on one of a pair of chromosomes is inherited by only half the offspring. But a gene drive allows a mutation made so that nearly all offspring will inherit the change. This spread can be rapid for species with short reproductive generation times and it can quickly sweep an edited gene through a population. This means that it will speed through a population exponentially faster than normal. A mutation engineered into a mosquito could spread through a large population within a season If that mutation reduced the number of offspring a mosquito produced, then the population could be wiped out, along with any malaria parasites it is carrying. The work is at an early stage, but such a technique could be used to wipe out disease-carrying mosquitoes or ticks, eliminate invasive plants or eradicate herbicide resistance (Komor *& al*, 2016).

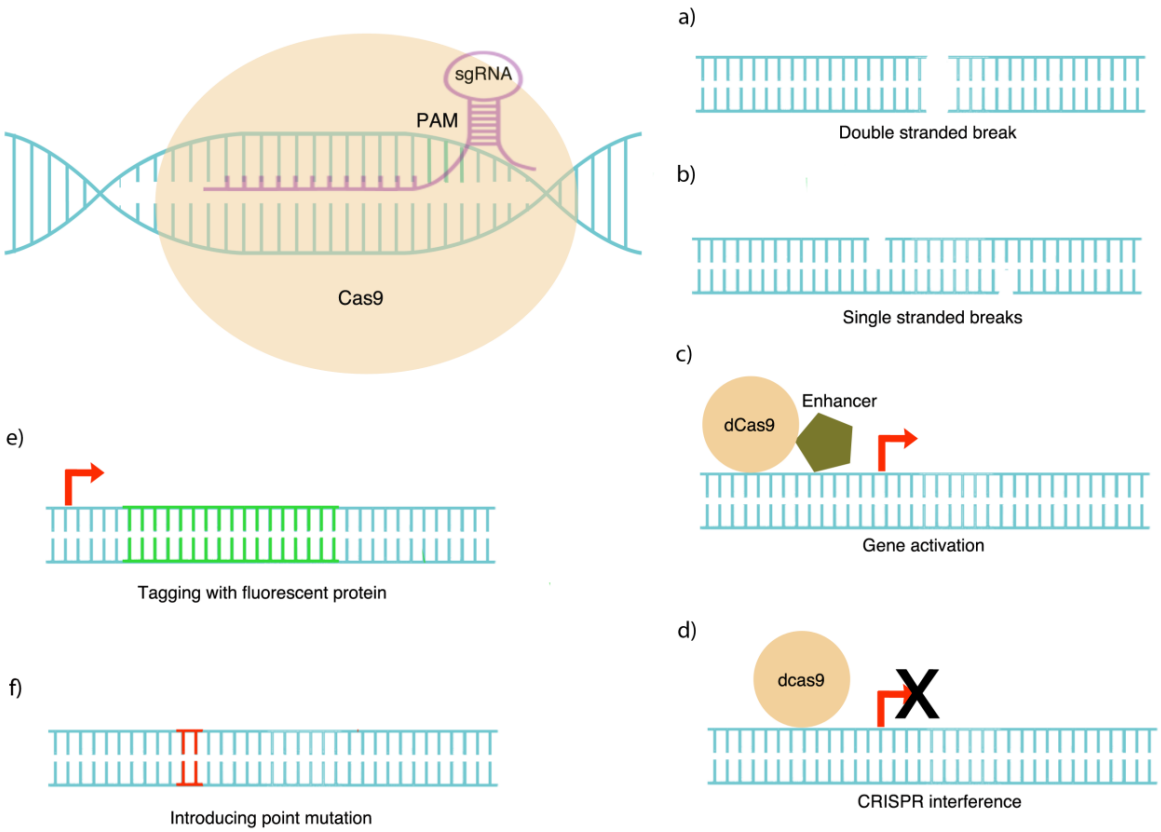


Figure 6: Overview of possible experimental designs of CRISPR/Cas9 (Ceasar *& al*, 2016)

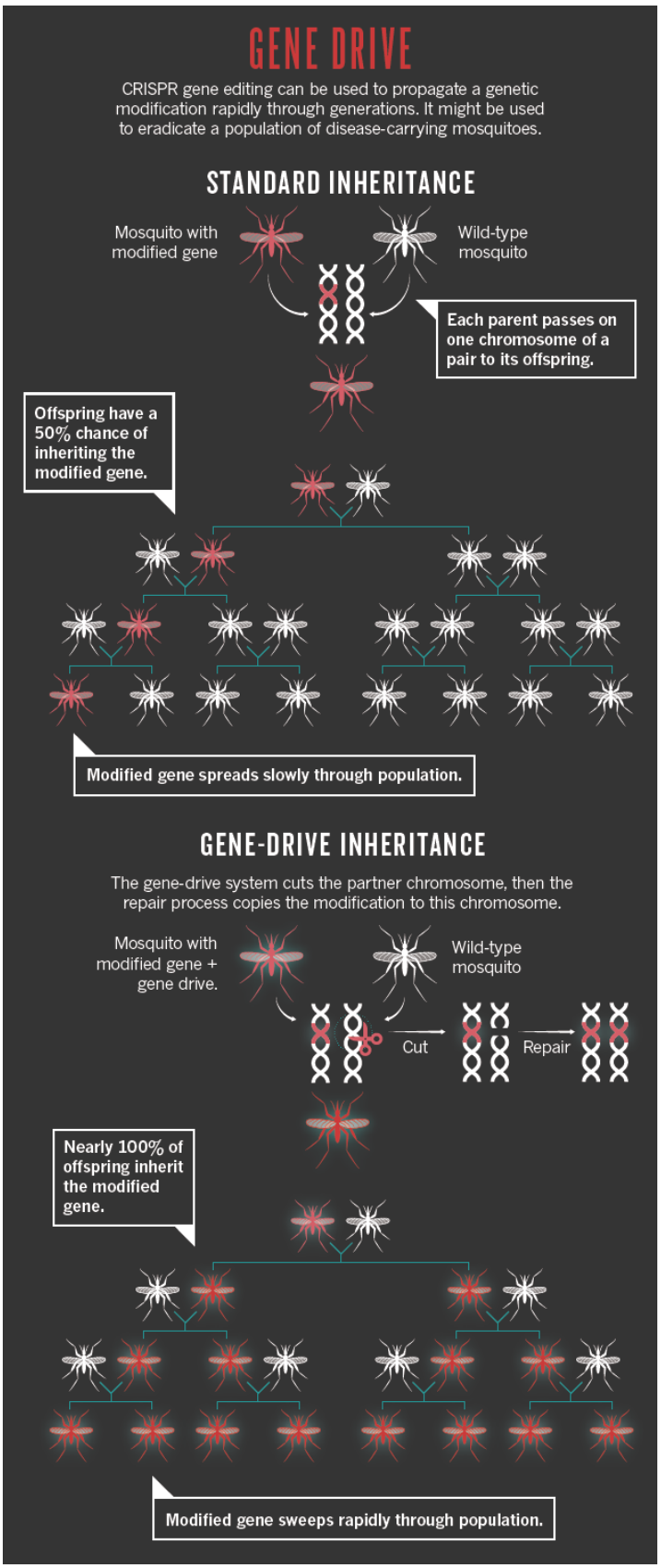


Figure 5: Gene drives (Ledford, 2015)

Chapitre II
Applications de CRISPR

Chapter 2 : CRISPR applications

CRISPR is incredibly powerful. It has already brought a revolution to the day-to-day life in most laboratories said the molecular biologist Jason Sheltzer. Programmable DNA cleavage using CRISPR–Cas9 enables efficient, site-specific genome engineering in single cells and whole organisms. It has been used in various ways, to alleviate genetic disorders in animals and is likely to be employed soon in the clinic to treat human diseases (Barrangou & Doudna, 2016). Of course, humans are not the only species with a genome. CRISPR has applications in animals and plants, too, from disabling parasites, to improving the crop yields.

Here, we take a look at the recent advances that demonstrate CRISPR's capabilities.

2.1. Disease fighter

2.1.1. Cancer

Cancer therapeutics arising from the CRISPR system as cited by multiple authors.

Cancer type	Modification	Contribution to therapy	Authors/(Refs.), year	Journal
Breast cancer	Knock-out of miR-644a	Inhibition of tumor growth, metastasis, and drug resistance	Raza *et al* (227), 2016	Oncotarget
Breast cancer	Knock-out (KO) BC200 lncRNA by CRISPR system	BC200 may serve as a prognostic marker and possible target for attenuating deregulated cell proliferation in estrogen-dependent breast cancer	Singh *et al* (228), 2016	Cell Death and Disease
Endometrial cancer	Knock-out of *MUC1* at cells by CRISPR system	Concomitant decrease of MUC1 and EGFR can be prognostic markers in human endometrial tumors	Engel *et al* (229), 2016	Oncotarget
Lung adenocarcinoma and endometrial carcinoma	Deletion of super-enhancers 3' to *MYC* in cells by using CRISPR system	Super-enhancers stimulate cancer driver genes in diverse types of cancer	Zhang *et al* (230), 2016	Nature Genetics
Endometrial cancer	*ERα*-null endometrial cancer cells	Inverse relationship between the tumor suppressor PR and the oncogene Myc in endometrial cancer	Kavlashvili *et al* (231), 2016	PLOS One
Prostate cancer	*NANOG* and *NANOGP8* knockout DU145 prostate	Attenuation of malignant potential of prostate cancer	Kawamura *et al* (232), 2015	Oncotarget
Prostate cancer	*NANOG* and *NANOGP8* knockout DU145 prostate cancer cell lines	Attenuation of malignant potential of prostate cancer	Kawamura *et al* (232), 2015	Oncotarget

Table 1: Cancer therapeutics arising from the CRISPR system (STELLA *& al*, 2018)

2.1.2. HIV

HIV still infects more than 35 million people worldwide according to the Global Health Observatory data (updated November 2017). Not only does the virus infect the very immune cells in the body that attack viruses, but it's also a notorious mutator. Currently, highly active antiretroviral therapy (HAART) is able to suppress HIV infection below detectable levels in HIV patients. However, HAART is limited in its high cost, patient compliance, side effects from long-term therapy, emergence of drug resistance. And most of all, it does not cure HIV infection. Therefore, there is a continuous need to develop more effective therapeutics and cure strategies for HIV infection (Yin *& al*, 2018). Researchers now are using CRISPR to snip the virus from the cell it was infecting, shutting down the virus's ability to replicate. The first use of the CRISPR technique to eradicate HIV virus was in 2017 where they have demonstrated a way to eliminate HIV from infected cells, resulted in a reduced viral RNA expression and successful proviral excision (Yin *& al*, 2017). One of techniques used was inhibiting the gene coding sequences which resulted in a strong inhibition of HIV-1 by Cas9/gRNA. This inhibition was obtained due to the deletions that were introduced into viral DNA due to Cas9 cleavage in the cytoplasm and in the nucleus (Yin *& al*, 2018).

2.1.3. Cystic Fibrosis

Cystic fibrosis is a progressive, genetic disease that originates from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) in the gastrointestinal and pulmonary tract. the mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene cause the CFTR protein which is an ion channel that regulates epithelial fluid transport to become dysfunctional. Loss-of-function alleles lead to an accumulation of mucus (a thick and sticky mucus) in the gastrointestinal and pulmonary tract, causing a number of symptoms such as difficulties in breathing and recurrent infections. In the lungs, the mucus clogs the airways and traps germs, like bacteria, leading to infections, inflammation, respiratory failure, and other complications (Savić & Schwank, 2015). It was recently shown that the cystic fibrosis transmembrane conductor receptor (CFTR) gene can be corrected by the use of CRISPR-Cas9. The obtained results were possible by targeting the CFTR gene in cultured intestinal stem cells isolated from cystic fibrosis patient (Schwank *& al*, 2013).

2.1.4. Huntington disease

A fatal genetic neurodegenerative disorder that causes nerves in the brain to deteriorate over time. The condition results from a faulty gene that becomes larger than normal and produces a larger-than-normal form of a protein called huntingtin. The elongated protein is cut into smaller, toxic fragments that bind together and accumulate in neurons, disrupting the normal functions of these cells. This process particularly affects regions of the brain that help coordinate movement and control thinking and emotions.The dysfunction and eventual death of neurons in these areas of the brain underlie the signs and symptoms of Huntington disease. Although suppressing the expression of mutant HTT (mHTT) has been explored as a therapeutic strategy to treat Huntington's disease (Shin *& al*, 2016). Considerable efforts have gone into developing allele-specific suppression of mHTT expression. Researchers reported that they have reversed the disease by permanent suppression of endogenous mHTT expression in lab mice that had been engineered to have a human mutant huntingtin gene in place of a mouse huntingtin gene. Used CRISPR/Cas9-mediated inactivation effectively to snip out part of the mutant huntingtin gene that produces the toxic bits resulted in evacuating the HTT aggregates and attenuated early neuropathology. The reduction of mHTT expression in neuron cells in adult mice did not affect viability, but alleviated motor deficits. This study suggested that CRISPR/Cas9-mediated gene editing could be used to efficiently and permanently eliminate the gene expansion–mediated neuronal toxicity in the adult brain and the obtained results showed the potential of CRISPR to help fight this condition (Yang *& al*, 2017).

2.2.Mutation corrector

2.2.1. Blindness

One of the most common causes of childhood blindness is a condition called Leber congenital amaurosis, which affects about 2 to 3 per 100,000 newborns, LCA is a group of inherited retinal diseases causing blindness or severe vision loss in early childhood. The condition is caused by mutations that lead to the degeneration and/or dysfunction of photoreceptors, the cells in the retina that make vision possible. Photoreceptors capture light, converting it to electrical signals which are sent to the back of the brain to create the images we see. Mutations in one of more than two dozen genes can cause LCA (Foundation Fighting Blindness , 2017). A study explored the potential of CRISPR/Cas9-mediated gene editing to correct the mutation. Where it showed that the use of the CRISPR system could efficiently correct the mutation by deletion of the mutant genes. These researchers used the CRISPR-Cas strategy as a treatment and they noticed a

remarkable reduction in the number of mutant cells. These results showed the therapeutic potential of CRISPR-Cas strategies in treating patients with LCA (Ruan *& al*, 2017).

2.2.2. Duchenne Muscular dystrophy

Duchenne muscular dystrophy (DMD) is a debilitating condition that develops because of a mutation in of the longest genes in the body, which codes for the dystrophin protein. Because of the mutation in the dystrophin gene, the body doesn't make a functional form of the protein dystrophin, which is essential for muscle fiber health and for the integrity of muscle cell membranes of striated muscles. Over time, the lack of this protein causes progressive muscle degeneration and weakness.There is no effective treatment for this disease. Numerous approaches to rescue dystrophin expression in DMD have been attempted. However, these approaches cannot correct *DMD* mutations or permanently restore dystrophin expression (Pichavant *& al*, 2011).

In April 2017, a team of researchers used CRISPR to find ways to fight Duchenne muscular dystrophy. They had used a variation of the CRISPR tool, called CRISPR-Cpf1 (CRISPR from *Prevotella* and *Francisella),* to correct the mutation that causes Duchenne muscular dystrophy. They fixed the gene in human cells growing in lab dishes and in mice carrying the defective gene by deletion of some of the Exon in that mutated gene. They showed that Cpf1 provides a robust and efficient RNA-guided genome editing system that can be used to permanently correct *DMD* mutations by different strategies, thereby restoring dystrophin expression and preventing progression of the disease. These findings showed the efficiency of Cpf1-mediated correction of genetic mutations in human cells and an animal disease model and represent a significant step toward therapeutic translation of gene editing for correction of DMD (Zhang *& al*, 2017).

2.2.3. Fanconi Anemia

Fanconi anemia (FA) is a rare genetic disorder with several subtypes of FA that result from the inheritance of two gene mutations in each of at least 18 different genes. It is classified in the category of inherited bone marrow failure syndromes and it is a condition that affects many parts of the body such as small sized babies at birth, abnormal thumbs and/or radial bones, skin pigmentation, small heads, small eyes, abnormal kidney structures, and cardiac and skeletal anomalies. People with this condition may have bone marrow failure with a progressive deficiency of all bone marrow production of blood cells, red blood cells, white blood cells, and

platelets, physical abnormalities, organ defects, and an increased risk of certain cancers such as the acute myeloid leukemia (AML) (Fanconi Anemia, 2014).

Cas9-mediated genome editing has been used to correct the mutations in this disease where some researchers employed fibroblasts derived from a patient with Fanconi anemia as a model to test the ability of the CRISPR/Cas9 nuclease system to mediate gene correction. They showed that the Cas9 nuclease and nickase each resulted in gene correction, but the nickase, because of its ability to preferentially mediate homology-directed repair, resulted in a higher frequency of corrected genes (Osborn *& al*, 2015).

These studies collectively demonstrate significant progress toward developing treatments for genetic diseases. Nearly all genetic diseases and even potential cures for them (Komor *& al*, 2016).

2.3.Life improvement

2.3.1. Agricultural applications

Just as CRISPR can be used to modify the genomes of humans and animals, in 2013, its application in plants was successfully achieved. This breakthrough has opened up many new opportunities for researchers, including the opportunity to gain a better understanding of plant biological systems more quickly (Barrangou & Doudna, 2016).

The use of CRISPR/Cas systems covers various applications, from biotic stress tolerance to abiotic stress tolerance, and also includes the achievements of improved yield performance, biofortification and enhancement of plant quality by reducing disease in some crops and makes others more robust. The most important group of target applications relates to yield traits followed by the achievement of biotic or abiotic stress tolerance. Biotic stress tolerance includes induced tolerance to viral, fungal and bacterial diseases. As for abiotic stress tolerance, the two main objectives are to achieve herbicide and natural environmental stress tolerances. Environmental stress includes cold, salt, drought and nitrogen stress. All of these trait improvements are related to economic and agronomic challenges faced by farmers as pathogens, and environmental conditions are important threats that need to be dealt with in agriculture. Furthermore, plant breeders are continually trying to increase yield performances. The most studied crop is rice (Oryza sativa) followed by other major crops: maize (Zea mays), tomato (S. lycopersicum), potato (Solanum tuberosum), barley (Hordeum vulgare) and wheat (Triticum aestivum).

Humans have been improving the yield and disease resistance of crops and to ameliorate the quality and quantity of nutrition for hundreds of years through traditional agricultural methods

which are based on Lucky hits. But now scientists used CRISPR to do genetic modification of food plants, which is known in the world of science as GMOs (genetically modified organisms) those organisms, are created in laboratory using genetic engineering techniques. Those techniques consist of removing one or more genes from the DNA of another organism, such us bacterium, virus, animal, or plant and "recombine" them into the DNA of the plant they want to alter. By adding these new genes, genetic engineers hope the plant will express the traits associated with the genes. For example, genetic engineers have transferred genes from a bacterium known as BT into the DNA of corn. BT genes express a protein that kills insects, and transferring the genes allows the corn to produce its own pesticide Ricroch *& al*, 2017).

2.4. Editing human embryos

The speed with which CRISPR-based studies can go from hypothesis to result is astounding. Experiments that used to take months now take weeks. And since these experiments were done on different species, they tried to apply some on early human embryos. In 2107, a report issued by the National Academies of Sciences and Medicine recommends that trials on human embryos can be done but under certain conditions and altering the cells in embryos, eggs and sperm was ethically permissible provided that it was done to correct a disease or a disability, not to enhance a person's physical appearance or abilities but to prevent genetic diseases from being passed to future generations (Saey, 2017).

More than 10,000 inherited disorders have been identified, affecting millions of people worldwide and often there are no cures for these diseases. Correcting disease-causing genetic defects in human zygotes was previously unthinkable because the efficiency would be too low to be of any practical value. CRISPR/Cas9 offers, for the first time, a tangible potential to allow the correction of genetic defects (Tang *& al*, 2017). Among these diseases there are autosomal dominant mutations, where inheritance of a single copy of a defective gene can result in clinical symptoms. Genes in which dominant mutations manifest as late-onset adult disorders include *MYBPC3*, mutation of which causes hypertrophic cardiomyopathy (HCM). Because of their delayed manifestation, these mutations escape natural selection and are often transmitted to the next generation. HCM is a myocardial disease characterized by left ventricular hypertrophy, myofibrillar disarray and myocardial stiffness; it has an estimated prevalence of 1:500 in adults and manifests clinically with heart failure. HCM is the commonest cause of sudden death in otherwise healthy young athletes. HCM, while not a uniformly fatal condition, has a tremendous impact on the lives of individuals, including physiological (heart failure and arrhythmias), psychological (limited activity and fear of sudden death), and genealogical concerns. MYBPC3 mutations account for approximately 40% of all genetic defects causing HCM and are also

responsible for a large fraction of other inherited cardiomyopathies, including dilated cardiomyopathy and left ventricular non-compaction6. MYBPC3 encodes the thick filament-associated cardiac myosin-binding protein C (cMyBP-C), a signalling node in cardiac myocytes that contributes to the maintenance of sarcomeric structure and regulation of both contraction and relaxation. Recent developments in precise genome-editing techniques and their successful applications in animal models have provided an option for correcting human germline mutations. In particular, CRISPR–Cas9 where it was used in different division phases in a zygote carrying the previous MYBPC3 mutation. The injection of CRISPR-Cas9 during S-phase resulted in mosaic embryos consisting of non-targeted mutant, targeted NHEJ-repaired and targeted HDR-repaired blastomeres (Figure 6). These trials confirmed the efficiency, accuracy and safety of the CRISPR-Cas9 mediated-gene correction and suggest that it has potential to be used for the correction of heritable mutations in human embryos. However, much remains to be considered before clinical applications, including the reproducibility of the technique with other heterozygous mutations (Ma *& al*, 2017).

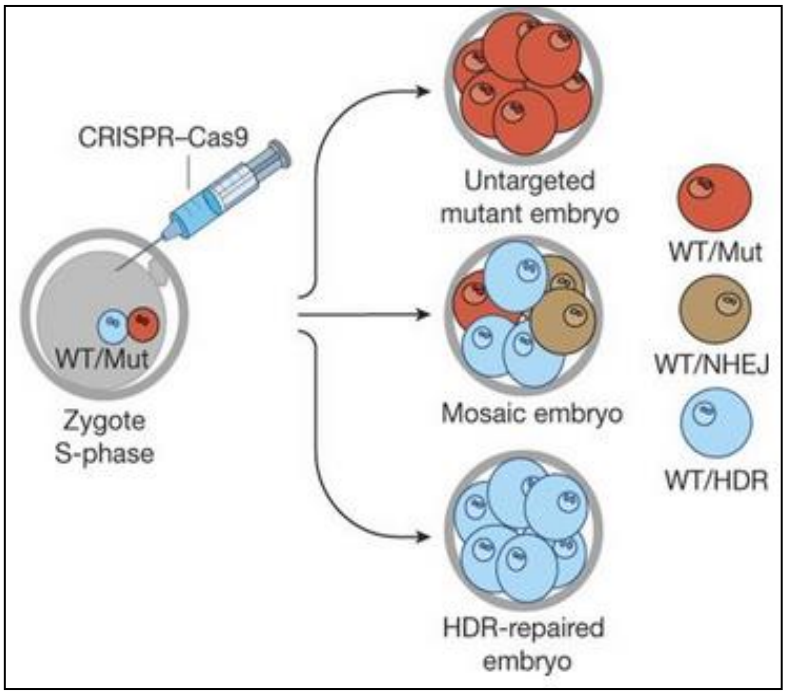
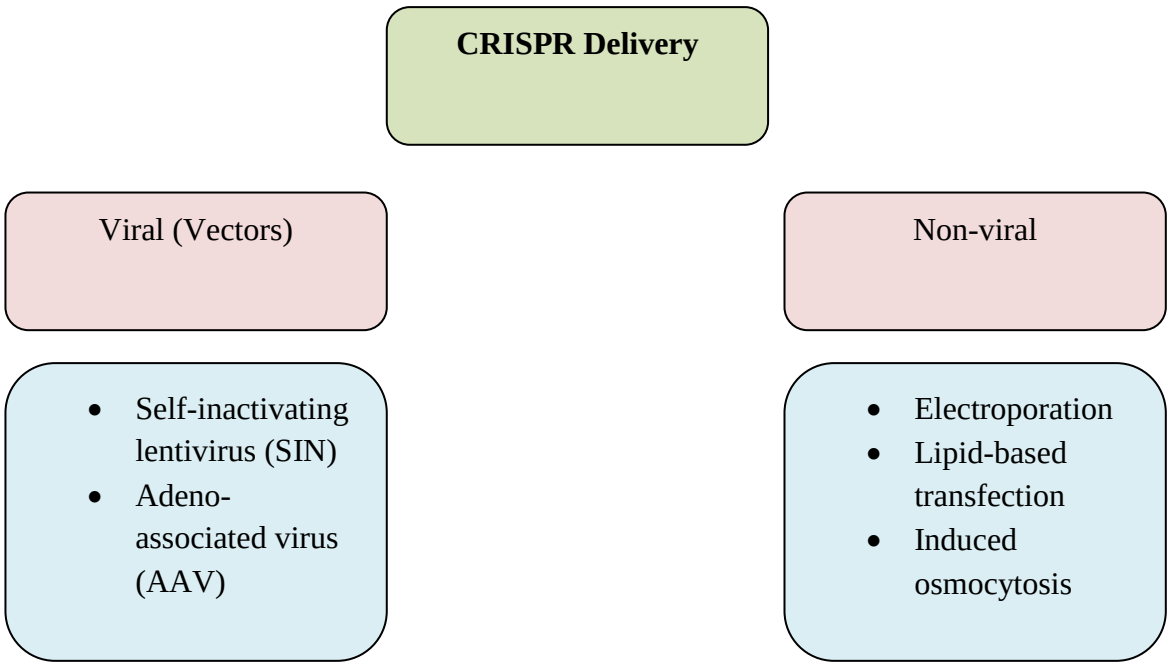


Figure 6: Gene correction in S-phase-injected human embryos (Ma *& al*, 2017).

2.5. **Delivery of CRISPR/Cas**

To get the maximum out of CRISPR/Cas9 technologies, along with refining their precision and efficiency, the delivery methods used need to be improved. Delivery methods differ depending on the cell type and application approach. CRISPR delivery methods (Therapy, 2015):



Chapitre III
Potentiels de CRISPR

Chapter 3 : Potentials of CRISPR

3.1. CRISPR-Cas as a tool for drug discovery

The (CRISPR/Cas9) system is triggering a revolution in the field of biology. And the trials done with CRISPR led to improving the standard care of patients with different diseases. Such advances have stimulated interest in 'personalized' or 'precision' medicine, which combines classical patient information with personal genetic data to directly inform individual treatment strategies. In addition to generating powerful research tools, genome editing with CRISPR–Cas technology holds great promise to make therapeutic agents or as a therapeutic itself, because the unmet medical needs for numerous diseases and the rapid progress of CRISPR-Cas gene editing can feed into a drug discovery (Fellmann *& al*, 2016). And from numerous models we chose the CAR T cells-based therapies.

3.1.1. Chimeric antigen receptor (CAR) T cells-based adoptive immunotherapy

The application of genome editing for therapeutic purpose has begun to overlap with the rapidly evolving field of cancer immunotherapy, particularly for the production of next-generation chimeric antigen receptor (CAR) T cells. These modified T cells armed with tumour-targeting receptors have demonstrated great promise in clinical trials treating various leukaemias and lymphomas and may eventually be used to treat solid cancers.

Chimeric antigen receptor (CAR) T cells therapy is a personalized immuno-oncology treatment. The CARs comprise **(Figure 7):**

- An extracellular binding domain which recognizes an antigen that is strongly expressed on – and specific to – tumor cells.
- An intracellular chimeric signaling domain that activates the T cell upon receptor engagement and promotes T cell-mediated killing of tumor cells (Ren & Zhao, 2017).

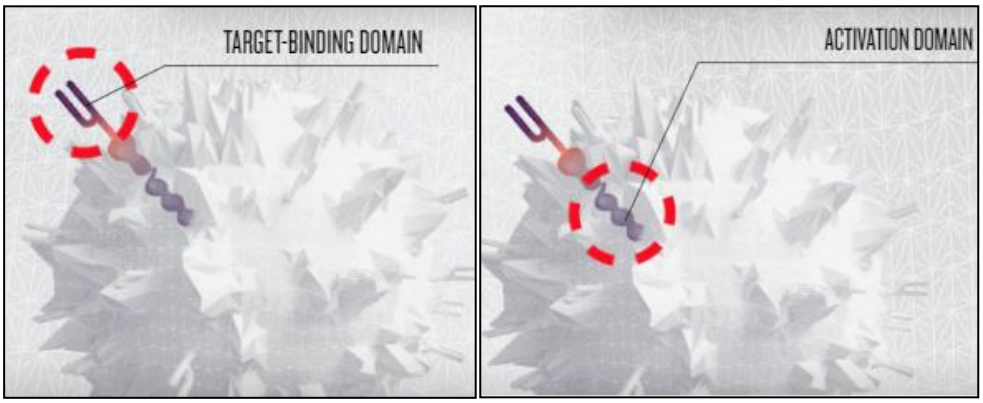


Figure7: CAR T cells domains (science, 2017)

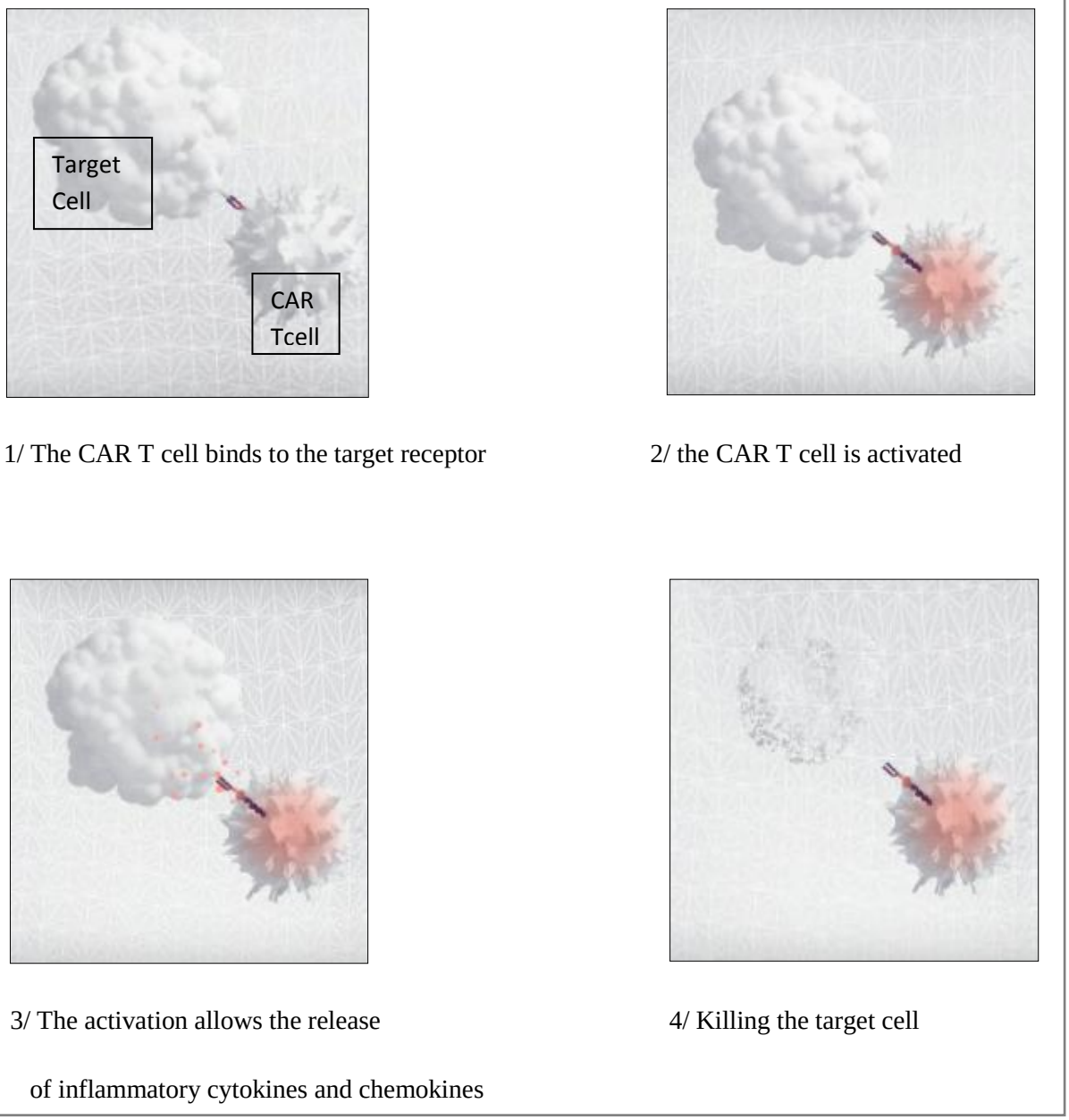


Figure 8 : Mechanism of action of CAR T cells (science, 2017)

Most of CAR T cells are generated by using each patient's own T cells(expensive and time consuming process that involves: isolating, modifying and expanding T cells for every new patient).

The CAR T cells therapy could become much faster and less expensive if universal donor CAR T cells could be generated as "off-the-shelf" cells (off-the-shelf: means a product that is available immediately and does not need to be specially made to suite a particular purpose) which would increase he number of patients that could be treated by single CAR T cell product. However, the major barrier to an off-the-shelf approach is host rejection caused by the mutual recognition of the CAR T cells and he host cells.

Genome editing strategies could also be used to prevent or delay the rejection of CAR T cells by the recipient's immune system through the elimination or he decrease in the expression of histocompatibility antigens on the donor T cells. And from this theory, scientists used CRISPR-Cas9 to disrupt the genes of T-cells in order to make off-the-shelf T cells(TCR disruption). However, the results showed that:

- the CAR T cells were still donor dependant
- the CAR T cells xere resistant to host rejection

thus highlighting the ability to generate multi-functional universal CAR T cells with CRISPR-Cas9 techniques (Ren & Zhao, 2017).

3.2.Blood disorders

CRISPR-Cas system has revolutionized methodologies in hematology and oncology studies.
This technology can be used to remove and correct genes or mutations, and to introduce site-specific therapeutic genes in human cells. Inherited hematological disorders represent ideal targets for CRISPR-Cas9-mediated gene therapy. Correcting disease-causing mutations could alleviate disease-related symptoms in the near future. From correcting non-cancerous hematological disorders like the β-thalassemia (an inherited disorder characterized by mutations in the human hemoglobin beta HBB; resulting in severe anemia). To ex-vivo trials on malignant hematological disorders like myeloma and leukemia which showed that this editing technique can be used in the near future, and will be extended to clinical treatments in patients (Zhang & McCarty, 2016).

3.3.Producing biofuels

Biofuels are a field of research that is expanding and reaching depths never before reached. Biofuels can be produced in a number of ways originating from living organisms or metabolic byproducts and contain over 80 percent renewable materials. The metabolic byproducts important in biofuel product are organic and food waste products. Biofuels provide many positives and are essential in shifting away from the usage of fossil fuels. They provide a high amount of energy security in that they are constantly available and sustainable which allows for a solid supply of affordable energy for different industries (Fairley, 2011).

3.3.1. CRISPR for gene regulation and succinate production in cyanobacterium

Cyanobacteria are a phylum of bacteria that obtain their energy through photosynthesis and are the only photosynthetic prokaryotes able to produce oxygen. Recent research has suggested the potential application of cyanobacteria to the generation of renewable energy by converting sunlight into electricity. And the direct conversion of CO2 into fuels, chemicals, and other value-added products. Recent engineering efforts have centered on harnessing the potential of these microbial biofactories for sustainable production of chemicals conventionally produced from fossil fuels (R.ShenChun *&* *al*, 2016).

Succinateis produced by the cyanobacteria and is widely used as a raw ingredient for petrochemicals while being seen as an alternative to replace the fossil-fuel sources. There is high demand for a way of producing succinate that is renewable and environmentally benign. Cyanobacterium *Synechococcus elongatus* PCC 7942 holds promise for biochemical conversion, but gene deletion in PCC 7942 is time-consuming and may be lethal to cells because of their nature. CRISPR-Cas9 is a being tried on these bacteria and CRISPR interference (CRISPRi) is the chosen technology to repress sequence specific genes without the need of gene knockout. These data demonstrated that CRISPRi-mediated gene suppression allowed re-directing the cellular carbon flow and enhanced the succinate concentration, thus paving a new avenue for the production of biotechnological products which can be used in the future as sustainable sources (Huang *&* *al*, 2016).

3.4. Resurrecting species

The feasibly of de-extinction varies among organisms, and not all organisms face the same technical challenges in their resurrection. For recently extinct species, it may be possible to use 'standard' cloning technology (such as the nuclear transfer followed by cellular reprogramming technique that most famously resulted in the birth of 'Dolly the Sheep' in 1996) and a closely related species as a surrogate maternal host (a substitute). Cloning via nuclear transfer has been accomplished for a wide range of mammalian species. However, If extinction occurred before living tissues could be collected and preserved, then, cloning is not possible because DNA decay begins immediately after death. The first step to resurrecting long-extinct species is therefore to sequence and assemble a genome from the preserved remains of that extinct species. The past decade has seen enormous advances in technologies for ancient DNA isolation and genome assembly, and high-quality genomes are now available for several extinct species, including mammoths and passenger pigeons, while this work is in progress for many other species. Once genome sequences are known, genome-wide scans can be used to create lists of genetic differences between the extinct species and their closest living relatives, which then become the initial targets for genome editing.

The successes of the different genome editing techniques demonstrate that genome editing using CRISPR/cas9 is feasible and efficient. The number of edits that would be required to turn, for example, an Asian elephant genome into a mammoth genome is not small; it is estimated that there are around 1.5 million nucleotide-level differences between these two species. However, the number of edits can be minimized by replacing large pieces of the genome in a single edit or by focusing on changing only those genes that are phenotypically relevant (Shapiro, 2015).

3.5. Diagnostic tool

With the epidemic of Lassa Fever in Nigeria early this year which was recorded on the track to be the worst ever recorded anywhere. Now, in the hope of reducing deaths from Lassa in years to come, researchers in Nigeria are trying out a new diagnostic test based on the gene-editing tool CRISPR. The test relies on CRISPR's ability to hunt down genetic snippets — in this case, RNA from the Lassa virus — that it has been programmed to find. If the approach is successful, it could help to catch a wide range of viral infections early so that treatments can be more effective and health workers can control the spread of infection. And scientists in different universities are testing CRISPR diagnostics for a wide range of viruses like dengue viruses, Zika viruses…

For most infectious diseases, diagnosis requires specialized expertise, sophisticated equipment and sufficient electricity , all of which are in short supply in many places where illnesses such as Lassa fever occur. The CRISPR tests offer the possibility of diagnosing infections as accurately as conventional methods, and almost as simply as an at-home pregnancy test. And because CRISPR is engineered to target specific genetic sequences, researchers hope to develop a tool

based on the technology that can be easy to identify, within a week, whatever viral strain is circulating.

Researchers at the Broad Institute of MIT and Harvard in Cambridge, who had paired CRISPR with the Cas13 protein. Unlike Cas9 (the enzyme originally used in CRISPR gene editing); Cas13 cuts the genetic sequence that it's been told to target, and then starts slicing up RNA indiscriminately. This behavior presents a problem when trying to edit genes, but it's a good for diagnostics because all that cutting can serve as a signal.

In 2018, the Broad team updated its test, called SHERLOCK, by adding RNA molecules that signal when they've been sliced by Cas13. The cut RNA triggers the formation of a dark band on a paper strip — similar to the visual cues in a pregnancy test — that indicates the presence of whatever genetic sequence CRISPR was engineered to find.

The team in Nigeria is now testing how accurately a version of this diagnostic, engineered to find the Lassa virus, on people whose infections have previously been confirmed with the polymerase chain reaction (PCR).

SHERLOCK is roughly half the price of PCR tests in Nigeria and takes half the time to return results (around two hours compared with four). Both diagnostics require electricity to process samples, but SHERLOCK isn't as sensitive to power outages -which are known in Nigeria- as PCR is.

Other CRISPR tests developed by other scientists use Cas proteins with different properties and patents to target various illnesses (Maxmen, 2019).

Conclusion

Conclusion

Genome editing is having a transformative effect in many areas of biological research. It is being taken up widely and has spread quickly due to the advantages it offers to those using it: it is affordable and easy to use; it gives faster results; it is efficient at making precise edits to DNA; and it offers the prospect of making these edits at multiple sites in the genome in a single procedure.

Summing it all up, CRISPR has its advantages and disadvantages ranging from ethical concerns to being known as the fastest, cheapest and most precise way of editing genes. This scientific breakthrough has the ability to eliminate diseases, solve world hunger, provide unlimited clean energy. CRISPR has potentially given us direct access to the source code of life and at the same time given a great amount of hope to billions of people (Doudna & Charpentier, 2014). The technology's promising ability to deliver is a major reason why so many investors are spending millions of dollars on it, in fact the sector CRISPR belongs to is experiencing something of a 'gold rush' due to all the dollar investments (Vezér, 2017). Adding on eighty percent of rare diseases are caused by faulty genes as mentioned in the Rare Diseases Organization. These figures alone show what a huge impact the technology would have in our lives if used appropriately and for the right reasons. Success with the permanent implementation of the technology will definitely change the world and make it a better place to live in.

Adding on scientists are working day and night trying to reduce the errors that CRISPR brings along with it and in no time it will start being applied in a clinical setting. CRISPR has a much higher success rate than the other nuclease technologies when it comes to cutting DNA at the right place. When a technology is so revolutionary and so simple to use it cannot be bottled up therefore science will move forward so that humanity can benefit as a result. In summary, there are more proceedings yet to take place involving gene editing, being bigger guidance or debate as to the next step on gene editing or what is possible (Doudna & Charpentier, 2014).

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Résumé

CRISPR est un système de défense immunitaire découvert chez les bactéries et les prokaryotes. CRISPR signifie des séquences répétées régulièrement séparées par des espaceurs uniques non répétés.

Ces espaceurs uniques sont des fragments de sequences de virus et d'ADN invasif. Il est constitué d'un ARN guide et une protéine Cas qui est considérée comme ciseaux qui coupe l'ADN. CRISPR a un rôle de protecteur contre les invasions des pathogènes. Et il est utilisé dans les modifications génétiques d'ADN.

Mots clés : CRISPR /Cas, ADN, ARN, modification génétique, bactéries.

Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated system (Cas) is a system that provides immunity to the most of prokaryotic organisms against viral attacks and other foreign bodies.

Crispr systems consist of a scissor-like protein called Cas9 and a genetic GPS guide "The guide RNA". However, researchers reoriented and repurposed that primordial immune system to precisely manipulate genomes in most organisms by introducing DNA double- strand breaks at specific genome locations to introduce specific DNA modifications.

Keywords: CRISPR/Cas, DNA, RNA, genome engineering, bacteria.

ملخص

CRISPR هو نظام دفاعي مناعي اكتشف لدى البكتيريا و البدائيات . CRISPRعبارة عن تسلسلات متكررة مفصولة بانتظام عن طريق فواصل غير متكررة موجودة في الحمض النووي للبكتيريا

هذه الفواصل الفريدة عبارة عن شظايا من فيروسات و تسلسلات لأحماض نووية غازية .

يتألف هذا النظام المناعي من دليل و المتمثل في الحمض النووي الريبي و بروتين الذي يعتبر مقصا يقطع الحمض النووي.

CRISPR لديه دور وقائي للبكتيريا ضد الغزوات المرضية و يستخدم في التعديلات الوراثية لمختلف الأنواع الحيوانية.

الكلمات المفتاحية :

CRISPR , الحمض النووي, الحمض النووي الريبي, التعديل الوراثي, البكتيريا.