



## CRISPR Pioneers Win 2020 Nobel Prize for Chemistry

*\*Dariush D. FARHUD<sup>1,2,3</sup>, Marjan ZARIF-YEGANEH<sup>3,4</sup>*

1. School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Basic Sciences/Ethics, Academy of Medical Sciences, Tehran, Iran
3. Farhud Genetic Clinic, Tehran, Iran

4. Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

**\*Corresponding Author:** Email: farhud@tums.ac.ir

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### Abstract

Over the last few years, the development of genome editing has revolutionized research on the human genome. Recent advances in developing programmable nucleases, such as meganucleases, ZFNs, TALENs and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas, has greatly expedited the progress of gene editing from concept to clinical practice. The CRISPR has advantages over other nuclease-based genome editing tools due to its high accuracy, efficiency, and strong specificity. Eight years after CRISPR application for human genome edition by Emmanuelle Charpentier and Jennifer A. Doudna, the 2020 Nobel Prize in Chemistry has been jointly given to them for development of CRISPR-Cas9 gene editing, allows scientists to precisely cut and edit of DNA.

**Keywords:** Clustered regularly interspaced short palindromic repeats; Gene therapy; Nobel Prize

### Background

The Nobel Prize 2020 in Chemistry has been jointly awarded to **Emmanuelle Charpentier**

and **Jennifer A. Doudna** for their discovery of “a gene editing technology” (Fig. 1).



**Fig. 1:** **Emmanuelle Charpentier** (Max Planck Institute, Berlin, Germany) and **Jennifer Doudna** (University of California, Berkely, USA) share the 2020 Nobel chemistry prize for their discovery of gene-editing technique. After reference (1)

According to the Royal Swedish Academy of Sciences the technology that allows for precise changes to the genetic code of organisms has a "revolutionary impact on the life sciences" and "may make the dream of curing genetic diseases come true".

## Introduction

### *Jennifer Anne Doudna*

Jennifer Doudna is the biggest household name in the world of CRISPR, and for good reason - she is credited as the one who co-invented Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). She (born February 19, 1964) is an American Biochemist known for her pioneering work in CRISPR gene editing. Doudna was among the first scientists to propose that this microbial immunity mechanism could be harnessed for programmable genome editing. This tool effectively change the genetic makeup of any organism and fix a near-infinite number of problems. Doudna says "I am really kind of humbled" (2).

### *Emmanuelle Charpentier*

Emmanuelle Charpentier is the co-inventor of CRISPR. She was involved in the biochemical characterization of guide RNA and Cas9 enzyme-mediated DNA cleavage.

Her expertise in the fields of microbiology, biochemistry, and genetics helped pave the way for the discovery of CRISPR. She unexpectedly discovered what would form the basis of the technology within the immune system of *Streptococcus pyogenes*, one of the bacteria that cause the most harm to human, when she noticed that a molecule in its immune system was capable of disarming viruses by slicing up their DNA (2).

After publishing her findings in 2012 (3), Charpentier went on to collaborate with US Biochemist Jennifer A. Doudna. They were able to recreate the bacteria's genetic scissors in the lab, simplifying the molecular components. Charpentier, said she was "very emotional" after getting a call from Stockholm with the news.

So far only 56 women have won the Nobel Prize, instead of 817 men laureate (4) (Gender inequality!). As Rosalyn Franklin, who played a major role in discovering of the DNA molecule structure in 1953, was ignored by the Nobel Prize Committee, despite the emphasis and approval of James Watson.

Doudna and Charpentier and their colleagues did very mordant early work characterizing the system, but several other researchers have a key contributors in the development of CRISPR. They include Feng Zhang at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts (5); George Church at Harvard Medical School in Boston, Massachusetts (6), and biochemist Virginijus Siksnys at Vilnius University in Lithuania (7).

### *Other Scientists who have worked on CRISPR/Cas system*

Francisco Mojica: Discovered the Existence of CRISPR Sequence in Bacteria (8), Matthew Porteus: The Pioneer of Cell-Based CRISPR Therapies (9), David Liu: Introducing Base-Editor Enzymes (10), Stephen Tsang: Pioneer of Ophthalmological Genome Surgery Options (11), Kevin Esvelt: Changing the Game with Responsible Gene Drives (12), Prashant Mali: Taking the Multidisciplinary Approach (4), Stanley Qi: Developing the Next Stage of CRISPR Tech (13), Patrick Hsu: Expanding CRISPR Toolkit to RNA (14), Michel Sadelain: Novel Therapies Targeting Cancer (15), Jacob Corn: The Intersection of Genome Editing and DNA Repair (16), Alison Van Eenennaam: Using CRISPR to Edit Cattle (17).

### *Development of CRISPR Genome Editing Tool*

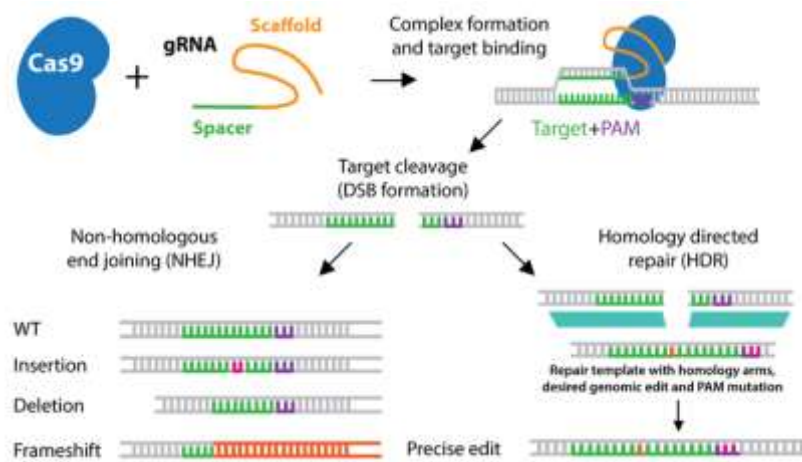
CRISPRs, are repeating DNA sequences in the genomes of bacteria and archaea and prokaryotes have long utilized CRISPR as a powerful defensive strategy against viral invaders. CRISPRs were first identified in *E. coli* in 1987 by a Japanese scientist, Yoshizumi Ishino (18), who accidentally cloned an unusual series of repeated sequences interspersed with spacer sequences while analyzing a gene responsible for the conversion of alka-

line phosphatase. However, the function of these sequences remained unclear, because of insufficient DNA data (19).

In 1993, researchers led by J.D. van Embden (20) in the Netherlands, discovered that different strains of *Mycobacterium tuberculosis* had different spacer sequences between the DNA repeats and were identified in several bacterial and archaeal genomes. For the first time, Francisco Mojica and Ruud Jansen mentioned these sequences as CRISPRs (8).

This prokaryotic immune system is used as a flexible genome engineering tool. This system is useful for research applications and influences other genome engineering technologies such as zinc finger nucleases (ZFNs) and transcription activator-like effectors (TALENs) (19).

The most frequently used subtype of CRISPR systems is the type II CRISPR/Cas9 system, which depends on a single Cas protein from *Streptococcus pyogenes* (SpCas9) (21) (Fig. 2).



**Fig. 2:** An overview of CRISPR and NHEJ/HDR. The Cas9/gRNA complex produces a DSB at the target genomic locus. Repair will proceed through NHEJ or HDR depending on the experimental conditions. After reference (19)

### **CRISPR/Cas Genome Engineering Mechanism**

The CRISPR/Cas9 system comprises of a single-stranded guide RNA (sgRNA) and a Cas9 endonuclease. The sgRNA (about 20 bp) designed to target DNA in a sequence-specific manner, and this must be followed by a short DNA sequence upstream essential for the compatibility with the Cas9 protein used, which is termed the “protospacer adjacent motif” (PAM) of an “NGG” or “NAG”. After sgRNA binding to the target sequence by Watson–Crick base pairing and Cas9 precisely cleaves the DNA to generate a double-strand break (DSB) and DNA-DSB repair mechanisms initiate genome repair including, non-homologous end joining (NHEJ), an error-prone process, or high-fidelity Homology-directed re-

pair (HDR) which introduces small insertions and deletions (indels) in the repair site (22).

Genome editing with CRISPR is not only simple, but also scalable. Multiple gRNAs targeting multiple loci can be easily expressed in the same cell or organism. The ability to precisely target CRISPR to a given locus makes it especially amenable to genetic screens (19).

CRISPR Applications (19):

Scientists use the targeting capability of CRISPR to make modifications at specific loci, including:

- Knocking out of a specific gene (induced by NHEJ mechanism).
- Repairing disease-causing mutations (corrected by HDR mechanism).

- Activating or Repressing of a specific gene, dCas9 fused to transcriptional activators or repressors can modulate transcription in a reversible manner.
- Epigenetics, dCas9 fused to epigenetic modifiers can modulate transcriptions and create heritable epigenetic marks.
- Visualize: Fluorescently labeled dCas9 or gRNAs that bind fluorescent proteins can be used to image genomic loci in live cells.
- RNA
- Targeting, Cas13 enzymes target RNA rather than DNA, sometimes requiring a protospacer flanking sequence (PFS). In bacteria, Cas13 targeting also promotes non-specific RNA cleavage, but this non-specific cleavage does not occur in mammalian cells.
- Purify, Epitope-tagged dCas9 can also be used to purify a genomic locus and its associated proteins or RNAs through ChIP (enChIP) or biotin-streptavidin pulldown (CAPTURE).
- Tag: Multiple methods make it easier to tag endogenous loci with epitope tags or fluorescent markers.

### *The Future of CRISPR Directions*

CRISPR/Cas9 has emerged as an extremely powerful tool across many fields. The applications of this technology are limitless. Researchers are working on new ways to cure many diseases such as Cancer, Hereditary eye diseases, Cardiovascular disease, Metabolic diseases, diagnosis and treatment of Infectious diseases such as HIV and COVID-19 infections (CRISPR technology as an emerging pan-antiviral therapy) (23), neurodegenerative disorders such as Huntington (HD), Alzheimer, and Parkinson, Hematological diseases such as hemophilia, Sickle anemia,  $\beta$ -thalassemia, and Duchenne muscular dystrophy (DMD) (24).

Moreover, CRISPR/Cas9 gRNA library screening is an ideal tool for drug screening or the targeted screening of specific pathways. The generation of gRNA libraries will play an important role

in functional gene screening, disease mechanism research and drug development. CRISPR can also use as a Gene diagnostic tools, specifically for cancer gene mutation diagnosis (24).

Hopefully, there are many CRISPR gene editing clinical trials for treatment of human diseases that are in phases I/II (24).

Although CRISPR technology is a powerful tool for genetic studies, it has challenges in therapeutic approaches, therefore, scientists should consider many aspects of this new technology especially for human genome manipulations including, increasing the specificity of gene correction, improving the efficiency of nuclease editing, optimizing the delivery system.

Finally, it is our honor that since 2017 our group and some other scientists in Iran has started the CRISPR studies in various fields, successfully (25-27).

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### **Conflict of interest**

The authors declare that there is no conflict of interests.

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