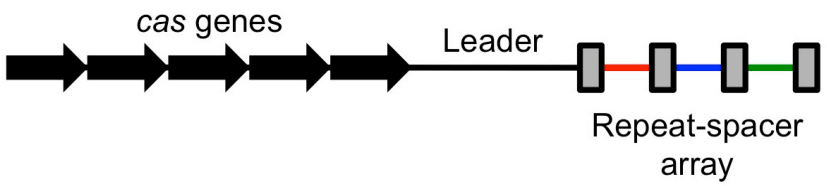
CRISPR Technology-
The Discovery,TheTechnology and its Implications

SUMAN UPADHYAY
MSC STUDENT IN BIOLOGY

Introduction

- **Clustered regularly-interspaced short palindromic repeats** (abbreviated as **CRISPR** are segments of prokaryotic DNA containing short repetitions of base sequences. Each repetition is followed by short segments of "spacer DNA" from previous exposures to a bacterial virus or plasmid
- The **CRISPR/Cas system** is a prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages.
- The CRISPR interference technique has enormous potential application, including altering the germline of humans, animals and other organisms, and modifying the genes of food crops. By delivering the Cas9 protein and appropriate guide RNAs into a cell, the organism's genome can be cut at any desired location.CRISPRs have been used in concert with specific endonuclease for genome editing and gene regulation in species throughout the tree of life

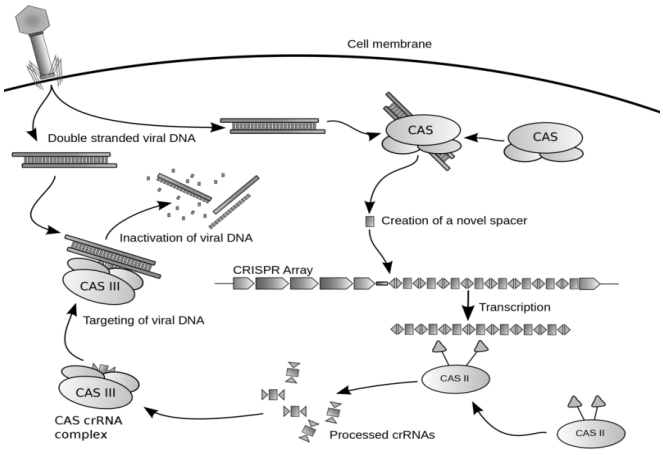


The three major components of a CRISPR locus are shown: *cas* genes, a leader sequence, and a repeat-spacer array. Repeats are shown as grey boxes and spacers are coloured bars. The arrangement of the three components is not always as shown. In addition, several CRISPRs with a similar sequences can be present in a single genome, only one of which is associated with cas genes

Cas9 protein

- Jennifer Doudna[1] and Emmanuelle Charpentier [2]found that bacteria respond to an invading phage by transcribing spacers and palindromic DNA into a long RNA molecule. The cell then uses tracrRNA and Cas9 to cut this long RNA molecule into pieces called crRNAs.

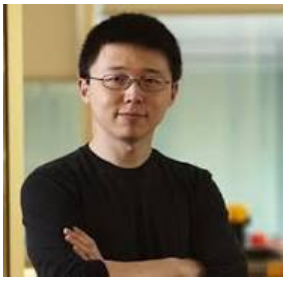
Diagram of the CRISPR prokaryotic viral defense mechanism



CRISPR interference

- **CRISPR interference** (CRISPRi) is a genetic perturbation technique that allows for sequence-specific repression or activation of gene expression
- Based on the bacterial genetic immune system - CRISPR (clustered regularly interspaced short palindromic repeats) pathway,the technique provides a complementary approach to RNA interference. The difference between CRISPRi and RNAi, though, is that CRISPRi regulates gene expression primarily on the transcriptional level, while RNAi controls genes on the mRNA level

Discovery of CRISPR interference-Contribution of Dr.Zhang(The New Yorker review)



Feng Zhang (born 1982) is the W. M. Keck Career Development Professor of Biomedical Engineering in the departments of Brain and Cognitive Sciences and Biological Engineering at the Massachusetts Institute of Technology. He also has appointments with theBroad Institute of MIT and Harvard (where he is a core member) and the McGovern Institute for Brain Research. He is most well known for playing a central role in the development of optogenetics and CRISPR technolog ies

Biography of Dr.Zhang

- Settled in Iowa with his parents in hope of a better education. His biological interest sparked at 13 as his mother advised him to attend a molecular biology class.
- In 1997, when Zhang was fifteen, he was offered an internship in a biosafety facility at the Des Moines Human Gene Therapy Research Institute—but he was told that federal law prohibited him from working in a secure lab until he was sixteen.So had to wait till his birthday when he finally got an opportunity to work and spend 5 hours everyday at the lab after school.

- He earned his A.B. in Chemistry and Physics from Harvard University in 2004 where he worked with Xiaowei Zhuang.
- He then received his Ph.D.in chemistry and bioengineering from Stanford University in 2009 under the guidance of Karl Deisseroth where he developed the technologies behind optogenetics with Edward Boyden

Dr.Zhang's research

- Zhang's lab is focused on using synthetic biology to develop technologies for genome and epigenome engineering to study neurobiology. As a postdoc, he began work on using TAL effectors to control gene transcription.
- Based on previous work by the Sylvain Moineau Lab, Dr. Zhang began work to harness and optimize the CRISPR system to work in human cells in 2011.
- While Zhang's group was optimizing the Cas9 system in human cells, the collaborating groups of Emmanuelle Charpentier and Jennifer Doudna reported a biochemical characterization of the CRISPR-Cas9 system, including the design of a single, chimeric guide RNA (sgRNA) capable of facilitating cleavage of DNA using purified Cas9 protein and sgRNA.
- Zhang's group further optimized this Doudna/Charpentier sgRNA design for expression in mammalian cells and subsequently reported the first application of Cas9 for genome editing in human cells the following year

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

[1]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA, and McGovern Institute for Brain Research, Department of Brain and Cognitive Sciences, Department of Biological Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA. [2]Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, MA 02115, USA. [3]Harvard-MIT Health Sciences and Technology, Harvard Medical School, Boston, MA 02115, USA. [4]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA. [5]School of Life Sciences, Tsinghua University, Beijing 100084, China. [6]Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA. [7]Computational and Systems Biology Graduate Program and Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [8]Laboratory of Bacteriology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

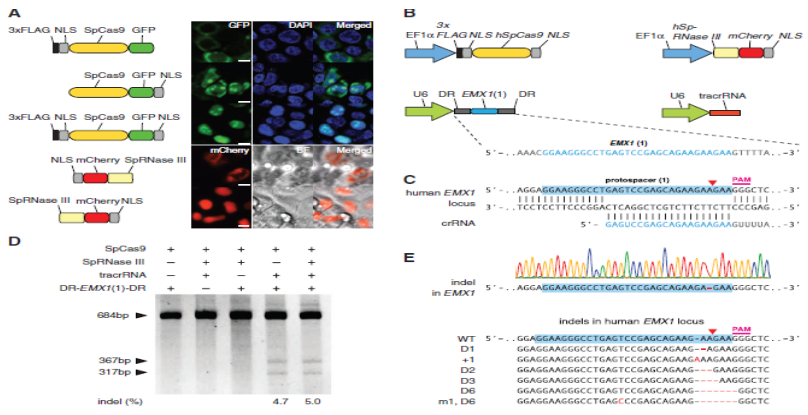


Fig. 1. The type II CRISPR locus from S. pyogenes SF370 can be reconstituted in mammalian cells to facilitate targeted DSBs of DNA. (A) Engineering of SpCas9 and SpRNase III with NLSs enables import into the mammalian nucleus. GFP indicates green fluorescent protein; scale/bars, 10 mm. (B) Mammalian expression of human codon–optimized SpCas9 (hSpCas9) and SpRNaseIII (hSpRNase III) genes were driven by the elongation factor 1a (EF1a) promoter, whereas tracrRNA and pre-crRNA array (DRSpacer-DR) were driven by the U6 promoter. A protospacer (blue highlight) from the human EMX1 locus with PAM was used as template for the spacer in the pre-crRNA array. (C) Schematic representation of base pairing between target locus and EMX1-targeting crRNA. Red arrow indicates putative cleavage site. (D) SURVEYOR assay for SpCas9-mediated indels. (E) (E) An example chromatogram showing a microdeletion, as well as representative sequences of mutated alleles identified from 187 clonal amplicons. Red dashes, deleted bases; red bases, insertions or mutations

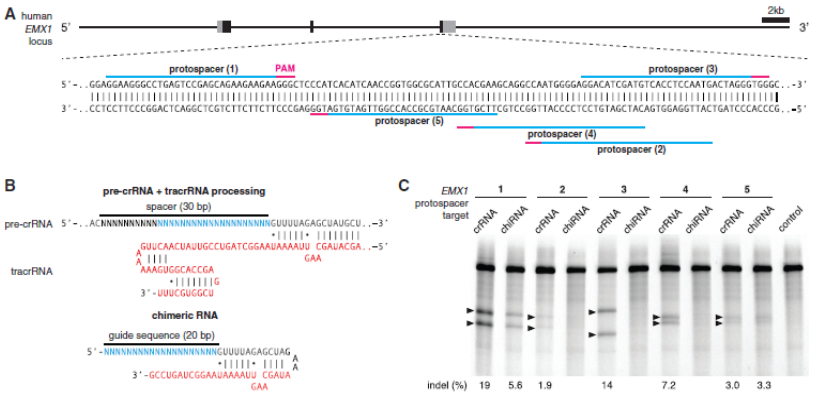


Fig. 2. SpCas9 can be reprogrammed to target multiple genomic loci in mammalian cells. **(A)** Schematic of the human *EMX1* locus showing the location of five protospacers indicated by blue lines with corresponding PAM in magenta. **(B)** Schematic of the pre-crRNA:tracrRNA complex (top) showing hybridization between the direct repeat (gray) region of the pre-crRNA and tracrRNA. Schematic of a chimeric RNA design (*12*) (bottom). tracrRNA sequence is shown in red and the 20-bp spacer sequence in blue. **(C)** SURVEYOR assay comparing the efficacy of Cas9-mediated cleavage at five protospacers in the human *EMX1* locus. Each protospacer was targeted by using either processed pre-crRNA:tracrRNA complex (crRNA) or chimeric RNA (chiRNA). Arrowheads indicate cleavage products for each protospacer target.

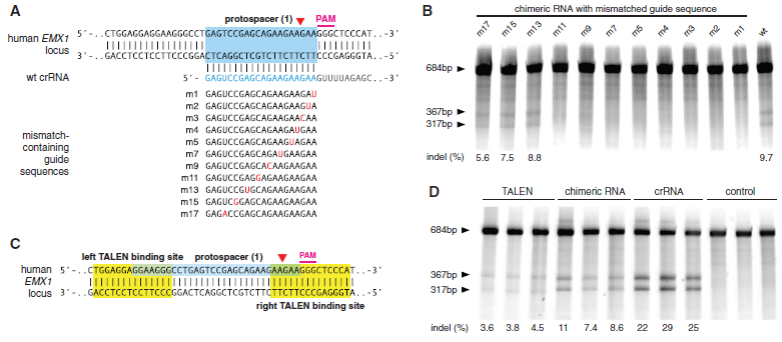


Fig. 3. Evaluation of the SpCas9 specificity and comparison of efficiency with TALENs. **(A)** *EMX1*-targeting chimeric crRNAs with single point mutations were generated to evaluate the effects of spacer-protospacer mismatches. **(B)** SURVEYOR assay comparing the cleavage efficiency of different mutant chimeric RNAs. **(C)** Schematic showing the design of TALENs that target *EMX1*. **(D)** SURVEYOR gel comparing the efficiency of TALEN and SpCas9 ($N = 3$).

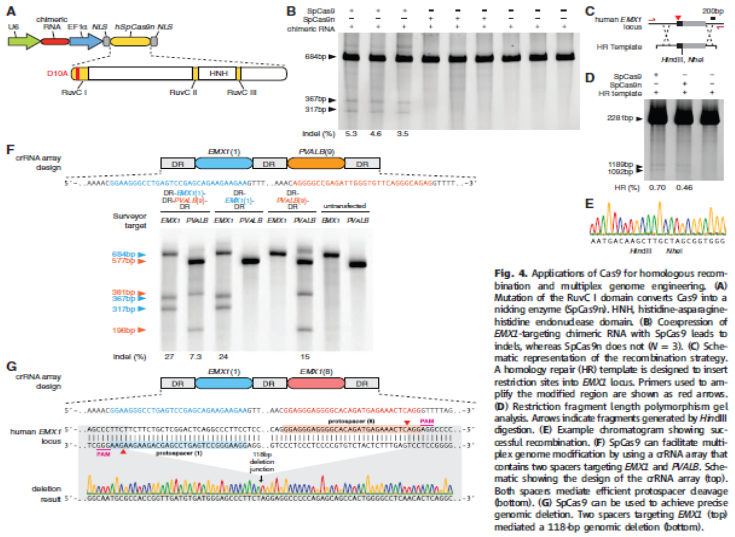


Fig. 4. Applications of Cas9 for homologous recombination and multiplex genome engineering. **(A)** Mutation of the RuvC I domain converts Cas9 into a nicking enzyme (SpCas9n). HNH, histidine-asparagine-histidine endonuclease domain. **(B)** Coexpression of *EMX1*-targeting chimeric RNA with SpCas9 leads to indels, whereas SpCas9n does not (*N* = 3). **(C)** Schematic representation of the recombination strategy. A homology repair (HR) template is designed to insert restriction sites into *EMX1* locus. Primers used to amplify the modified region are shown as red arrows. **(D)** Restriction fragment length polymorphism gel analysis. Arrows indicate fragments generated by *Hin*dIII digestion. **(E)** Example chromatogram showing successful recombination. **(F)** SpCas9 can facilitate multiplex genome modification by using a crRNA array that contains two spacers targeting *EMX1* and *PVALB*. Schematic showing the design of the crRNA array (top). Both spacers mediate efficient protospacer cleavage (bottom). **(G)** SpCas9 can be used to achieve precise genomic deletion. Two spacers targeting *EMX1* (top) mediated a 118-bp genomic deletion (bottom).

Conclusions from this paper

- *S.pyogenes* CRISPR system can be heterologously constituted in mammalian cells in genome editing thus efficient tool in genetic engineering

Dr.Zhang's honours

- Zhang is a recipient of the NIH Pioneer Award and a Searle Scholar. He was named one of MIT Technology Reviews's TR35 in 2013.
- His work on optogenetics and CRISPR has been recognized by a number of awards including: the 2012 Perl-UNC Neuroscience Prize (for optogenetics, shared with Boyden and Deisseroth).
- The 2014 Alan T. Waterman Award (for optogenetics and CRISPR-Cas9), the National Science Foundation's highest honor that annually recognizes an outstanding researcher under the age of 35
- The 2014 Jacob Heskel Gabbay Award in Biotechnology and Medicine (for CRISPR-Cas9, shared with Doudna and Charpentier);and the 2014 Young Investigator Award from the Society for Neuroscience (for optogenetics and CRISPR-Cas9)

Advantages

- **Advantages**
- 1. CRISPRi can silence a target gene of interest up to 99.9% repression.
- 2. Since CRISPRi is based on Watson-Crick base-pairing of sgRNA-DNA and an NGG PAM motif, selection of targetable sites within the genome is straightforward and flexible. Carefully defined protocols have been developed.
- 3. Multiple sgRNAs can not only be used to control multiple different genes simultaneously (multiplexing gene targeting), but also to enhance the efficiency of regulating the same gene target.
- 4. While the two systems can be complementary, CRISPRi provides advantages over RNAi. As an exogenous system, CRISPRi does not compete with endogenous machinery such as microRNA expression or function. Furthermore, because CRISPRi acts at the DNA level, one can target transcripts such as noncoding RNAs, microRNAs, antisense transcripts, nuclear-localized RNAs, and polymerase III transcripts. Finally, CRISPRi possesses a much larger targetable sequence space; promoters and, in theory, introns can also be targeted

Limitations

- **Limitations**
- 1.The requirement of a protospacer adjacent motif (PAM) sequence limits the number of potential target sequences. Cas9 and its homologs may use different PAM sequences, and therefore could theoretically be utilized to expand the number of potential target sequences.
- 2.Sequence specificity to target loci is only 14 nt long (12 nt of sgRNA and 2nt of the PAM), which can recur around 11 times in a human genome.Repression is inversely correlated with the distance of the target site from the transcription start site. Genome-wide computational predictions or selection of Cas9 homologs with a longer PAM may reduce nonspecific targeting.
- 3.Endogenous chromatin states and modifications may prevent the sequence specific binding of dCas9-sgRNA complex. The level of transcriptional repression in mammalian cells varies between genes. Much work is needed to understand the role of local DNA conformation and chromatin in relation to binding and regulatory efficiency

Types of cells that cen be edited

- Lyme disease infected cells
- Cancer cell lines
- Embryonic Stem cells
- Potential to cure cystic fibrosis
- HIV infected cells

Lot of potential in curing many types of diseases

Ethical concerns-Chinese innovation

- The ethical debate over the uses of CRISPR technology in humans was largely theoretical. Then a group at Sun Yat-sen University, in southern China, attempted to repair, in eighty-six human embryos, the gene responsible for betathalassemia, a rare but often fatal blood disorder.
- If those disease genes, and genes that cause conditions like cystic fibrosis, could be modified successfully in a fertilized egg, the alteration could not only protect a single individual but eventually eliminate the malady from that person's hereditary lineage.
- Given enough time, the changes would affect all of humanity. The response to the experiment was largely one of fear and outrage. The *Times* carried the story under the headline "CHINESE SCIENTISTS EDIT GENES OF HUMAN EMBRYOS, RAISING CONCERNS."
- Critics called the experiment irresponsible and suggested that the scientists had violated an established code of conduct.

What the Chinese were Trying

- But the Chinese researchers were not trying to create genetically modified humans. They were testing the process, and every CRISPR researcher I spoke to considered the experiment to have been well planned and carried out with extraordinary care.
- They took triploid zygotes"—a relatively common genetic aberration—"from I.V.F. clinics. They deliberately chose those because they knew no human could ever develop from them

- Fewer than half the embryos were edited successfully, and, of those, most retained none of the new DNA that was inserted into the genes. The experiment, which was published in the Beijing-based journal *Protein & Cell*, demonstrated clearly that the day when scientists could safely edit humans is far off. The CRISPR system also made unintended cuts and substitutions, the potential effects of which are unknown. In other cases, it made the right changes in some cells of the embryo but not in all of them, which could cause other problems

Why creating genetically modified babies are impossible?

- Nobody would try to employ CRISPR technology to design a baby, let alone transform the genetic profile of humanity, anytime soon. Even if scientists become capable of editing human embryos, it would take years for the genetically modified baby to grow old enough to reproduce—and then many generations for the alteration to disseminate throughout the population.
- Many scientists are confident enough to fix genetic alterations by reverse genetics in future years to come

REFERENCES

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THANK YOU