CRISPR/Cas9: The Brave New World of Genetic Engineering

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In the last decade, breakthroughs in genetic engineering have produced a new genetic editing tool called CRISPR/Cas9—a technology so robust that it can modify DNA sequences in virtually any living organism with incredible precision.[1-4] Adapted from an ancient microbial defense system, CRISPR/Cas9 can be programmed to remove, edit, and insert new genetic material—from large gene multiplexes to a single DNA base–pair.[2-5] CRISPR/Cas9 has been heralded for its simplicity, inexpensiveness, and array of applications in biology, biotechnology, and medicine.[1] The explosive rise of CRISPR/Cas9 in research and the media has yielded intense excitement and controversy worldwide.[2-5] As this genetic engineering revolution evolves faster than regulations to control it, the question becomes: how can we wield a technology powerful enough to remodel the human genome, yet simple enough to be performed by an undergraduate student?

The CRISPR/Cas9 saga began with the discovery of an ancient immune system that protects bacteria against infections by viruses.[2,6-7] Within a unique prokaryotic gene locus, small pieces of viral DNA were discovered to be incorporated into the bacterial genome.[8] This gene locus became known as Clustered Regularly Interspaced Palindromic Repeats (CRISPR).[8] It is now known that CRISPR–associated (Cas) genes function as an "adaptive" immune system that can sample viral DNA and integrate it into the CRISPR locus.[6] These stored pieces of viral DNA serve as a record of past infection to help bacteria detect and destroy this invader in future infections.[2,9] Upon reinfection, CRISPR combines an RNA template (derived from its own viral DNA copy) with CRISPR RNA and DNA–cleaving enzymes to create a "targeted missile" that scavenges the cell for viral DNA matching the template and subsequently destroys these foreign invaders by DNA degradation.[4,9]

What captivated researchers about this prokaryotic CRISPR/Cas system was its ability to make targeted double–stranded breaks (DSBs) in DNA at precise locations—for virtually any DNA sequence.[10,11]Previous research had shown that DSBs could stimulate genome editing by harnessing the natural ability of DNA to repair itself.[2,3] When a DSB occurs, DNA can detect and repair this break in two ways: by joining the split ends back together, or by introducing a new DNA fragment that is homologous to the sequences at each end of the break.[1,9] The "non–homologous" end joining (NHEJ) mechanism fuses DNA without any repair template. The error–prone NHEJ repairs DNA by inserting or deleting several base pairs while joining broken ends, leading to a frameshift mutation or gene knockout.[9,12] On the other hand, if an exogenous DNA repair template is present, DNA can instead use the homology–directed repair (HDR) mechanism to insert a foreign DNA segment.[9] Thus, if the RNA–guided CRISPR/Cas system could be modified to recognize a specific sequence of DNA, such as a genetic mutation, then a DNA break at this mutation site could stimulate genome editing through natural DNA recombination events.[1]

A significant breakthrough in 2012 was the invention of a tool that reprogrammed CRISPR/Cas9 to cut any desired DNA sequence in the prokaryotic genome.[13,14] This groundbreaking invention was credited to Jennifer Doudna and Emmanuelle Charpentier, who used the Cas9 protein with a CRISPR system derived from Streptococcus pyogenes to engineer a molecule that could split DNA at precise locations of their choosing.[1,13] Within months of this development, Siksnys et al. discovered that CRISPR/Cas9 could be applied to other prokaryotes, including S. thermophiles.[6] By January 2013, Feng Zhang of the Broad Institute was the first to adapt CRISPR/Cas9 to edit human cells, thus unlocking the potential of CRISPR/Cas9 in the eukaryotic genome.[15]

Though recombinant DNA technologies were first developed in the 1970s, CRISPR/Cas9 has transformed genetic engineering because of its precision, effectiveness, and relative affordability.[1-4] Other recent gene editing technologies like Zinc Finger Nucleases and transcription activator-like effector nucleases (TALEN) rely upon proteins to recognize DNA sequences, and have proven difficult to implement with precision.[10,16] On the other hand, CRISPR/Cas9 uses RNA–guided enzyme complexes to target DNA and achieve site–specific DNA cleavage, making it far more accurate and effective.[10,17] Rather than having to rebuild "hardware" each time scientists want to target a gene of interest, CRISPR/Cas9 acts like "software" that can easily be programmed and reprogrammed to target multiple genes.[2] Also, CRISPR/Cas9 is naturally multiplexable, meaning that CRISPR arrays can target multiple different DNA sequences simultaneously.[15]

The ease of programming CRISPR/Cas9 set the stage for a tidal wave of new genetic research.[4,18] In 2012, there were 126 papers published on CRISPR. Last year alone, this number reached 2,155 publications.[18] The ability to modify the genome, its epigenetic contexts, and its transcripts in eukaryotic cells has yielded a myriad of developments in basic science, biotechnology, and medicine.[1] Many researchers believe that CRISPR/Cas9 has the potential to cure many genetic diseases, starting with single–gene mutations like sickle–cell anemia and cystic fibrosis.[5] Using viral vectors, CRISPR/Cas9 can spread cell changes in vitro back to organisms in vivo.[10] For instance, a PCKS9 gene knockout using CRISPR/Cas9 introduced into the mouse liver was shown to lower blood cholesterol by 40%.[19]

The promise of CRISPR/Cas9 to edit human cells triggered a battle to commercialize the CRISPR/Cas9 technology.[18] Doudna and the team at UC Berkeley were first to file a CRISPR/Cas9 patent in May 2012, while Zhang and the Broad Institute of Harvard and MIT filed their initial patent claim in December 2012.[14,18] After Zhang adapted the CRISPR technology to eukaryotic cell lines, the Broad Institute filed 11 more patents claiming that it invented the CRISPR/Cas9 system for human use.[18] The Broad Institute then paid the U.S. Patent and Trademark Office (USPTO) to fast–track the review process on its claims. Many scientists were surprised when the USPTO began to approve Broad Institute patents in April 2014 before its decision on UC Berkeley's claim.[18]

A legal battle ensued between the researchers, their academic institutions, and private corporations who had already invested over a billion dollars into this CRISPR technology—all to answer the question, who owns CRISPR/Cas9?[18] In February 2017, the U.S. Patent Trial and Appeal Board ruled that the patent claims of the Broad Institute did not conflict with those of UC Berkeley, and the USPTO patent decisions would stand.[18] Thus, the UC Berkeley team would own the intellectual property and licensing rights for aspects of CRISPR/Cas9 in prokaryotic cells, while the Broad Institute won these rights in eukaryotic cells.[18]

With this patent decision, the Broad Institute would control the central patent for commercial uses of CRISPR in plant and animal cells, including agriculture and medicine.[14,18] While both groups agreed that academic institutions could freely conduct research with their technology, U.S. companies in biotechnology and pharmaceutical industries may have to pay licensing fees to both parties due to the nature of their patents.[18]

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Beyond commercial interests, the patent decision created divisions within the scientific community, since both parties desired the academic credit and prestige associated with invention of the CRISPR/Cas9 technology.[18] While the UC Berkeley and Broad Institute patent battle over CRISPR/Cas9 continues in Europe, it is estimated that over 860 CRISPR patents exist worldwide, with more added each day.[14,20]

Intellectual property aside, an imperative question is: who controls CRISPR–Cas9 research? Serious ethical issues have been raised by scientists and the public about advances in genetic engineering.[21] A mix of excitement and fear surrounds the possibility of gene therapy in humans to treat disease. But what if this technology is applied controversially, such as for human enhancement and creating "designer humans"?[2] In 2015, an international consortium of research organizations from the U.S., the U.K., and China called for a moratorium on making heritable modifications to the human genome.[21] Back in 1975, a moratorium on genetic engineering declared at a gene–editing summit in California was largely respected.[7] Yet, researchers and scientific groups have no regulatory authority to prevent misuse of CRISPR/Cas9.[7] Even if such regulatory mechanisms existed in one country, these regulations may differ from country to country, thus requiring cooperation on a global scale.

Since the CRISPR/Cas9 tool was developed five years ago, research advances in genetic engineering are far outpacing the policies and regulations surrounding this technology. In August 2016, human trials of CRISPR/Cas9–modified cells in lung cancer patients began at Sichuan University in China.[22] CRISPR/Cas9 co–inventor Doudna cautions that rushing the implementation of CRISPR/Cas9 in humans before its biochemical functions are optimized may lead to unintended consequences.[2] But the promise of lucrative profits and economic gain may override the calls of many scientists to proceed with caution. Research and Markets forecasts that the CRISPR and Cas genes market will reach over $4 billion USD by 2025.[23]

In a landmark February 2017 decision, the U.S. National Academy of Science and National Academy of Medicine announced its support for gene editing in viable human embryos.[24] This advisory group endorsed heritable gene alterations aimed to treat serious diseases where no reasonable alternatives exist.[24] The announcement came less than two years after the requested international moratorium on human gene editing. Just last year, scientists claimed that it would be "irresponsible to proceed [with human gene editing]" until the risks and societal impacts were better understood.[24] While the current endorsement limits the use of genetic engineering to therapeutic applications only, some worry that this decision will "open the floodgates" to controversial applications like eugenics in the future.

In March 2017, weeks after the US advisory group announced its support for therapeutic uses of human genetic engineering, a team of Chinese scientists published the first report of CRISPR/Cas9 manipulations in normal human embryos.[25,26] Published in Molecular Genetics and Genomics, this report documented a mixed success rate in treating mutations causing beta-thalassemia and favism in viable human embryos.[25,26] Meanwhile, other researchers are harnessing the CRISPR/Cas9 technology for experimental treatments to cure certain cancers, blindness, and other genetic conditions as early as this year.[24] While the topic of human embryo editing remains controversial, the scientific communities in most countries are still debating the ethical implications surrounding widespread applications of CRISPR/Cas9 in human gene therapies.[27]

CRISPR/Cas9 has launched scientists and society into a brave new world of genetic engineering. Adapted from an immune system of bacteria that has existed for billions of years,[6,10] CRISPR/Cas9 has been engineered into a genetic editing tool applicable in any organism.[1-2] While CRISPR/Cas9 shows promise in biotechnology, agriculture, and medicine, the ethical implications of this technology are hotly debated.[1,27] Should we use gene editing in traits that can be inherited and spread within the population? Where do we draw the line between prudent and frivolous uses of genetic engineering? The scientific community must acknowledge the responsibility that comes with the power to edit the human genome. Scientists and society must engage in democratic discourse on how best to harness the breakthrough that is changing humankind—macroscopically and microscopically.

References

1. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR–Cas9 for genome engineering. *Cell*. 2014 June;157(6):1262-1278.
2. Doudna J. How CRISPR lets us edit our DNA [Internet]. TED Ideas Worth Spreading; 2015 Oct [cited 2017 Feb 25]. Available from: https://www.ted.com/talks/jennifer_doudna_we_can_now_edit_our_dna_but_let_s_do_it_wisely/transcript?language=en
3. Doudna J. Jennifer Doudna (UC Berkeley/HHMI): Genome Engineering with CRISPR–Cas9 [video on the Internet]. 2015 March 23 [cited 2017 Feb 25]. Available from: https://www.youtube.com/watch?v=SuAxDVBt7kQ
4. Jorgensen E. What You Need to Know About CRISPR [video on the Internet]. 2016 Oct 24 [cited 2017 Feb 25]. Available from: https://www.youtube.com/watch?v=1BXYSGepx7Q
5. Kahn J. Gene Editing Can Now Change an Entire Species—Forever [video on the Internet]. 2016 May [cited 2017 Feb 25]. Available from: https://www.ted.com/talks/jennifer_kahn_gene_editing_can_now_change_an_entire_species_forever/transcript?language=en
6. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc Natl Acad Sci* USA. 2012 September 25;109(39):E2579-86.
7. CRISPR Update. CRISPR Timeline [Internet]. Advanced Analytical Technologies Inc [cited 2017 March 2]. Available from: http://www.crisprupdate.com/crispr-timeline/
8. Mojica FJ, Díez–Villaseñor C, García–Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol*. 2005;60:174–182.
9. Greb C. Gene Editing with CRISPR/Ca9 – Breakthrough in Genome Engineering [Internet]. Leica Microsystems; 2016 November 17 [cited 2017 February 28]. Available from: http://www.leica-microsystems.com/science-lab/crispr-cas/gene-editing-with-crisprcas9-breakthrough-in-genome-engineering/
10. Zhang F. Dr. Feng Zhang speaks at the Canada Gairdner Symposium: RNA and the New Genetics [video on the Internet]. 2015 November 24 [cited 2017 February 25]. Available from: https://www.youtube.com/watch?v=yT5JeLVUgdo
11. Broad Institute. CRISPR Timeline [Internet]. Broad Institute [cited 2017 Feb 28]. Available from: https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline
12. Gong C, Bongiorno P, Martins A, Stephanou NC, Zhu H, Shuman S, et al. Mechanism of nonhomologous end–joining in mycobacteria: a low-fidelity repair system driven by Ku, ligase D and ligase C. *Nat Struct Mol Biol*. 2005 April;12(4):304-12.
13. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual–RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012 August 17;337(6096):816-21.
14. Ledford H. Titanic Clash Over CRISPR Patents Turns Ugly [Internet]. Nature News; 2016 September 21 [cited 2017 March 2]. Available from: http://www.nature.com/news/titanic-clash-over-crispr-patents-turns-ugly-1.20631
15. Cong et al. Multiple genome engineering using CRISPR/Cas systems. *Science*. 2013 February;339(6131):819-23.
16. Gaj T, Gersbach CA, Barbas III CF. ZFN, TALEN and CRISPR/Cas–based methods for genome engineering. *Trends Biotechnol*. 2013 July;31(7):397-405.
17. O'Connell MR, Oakes BL, Sternberg SH, East–Seletsky A, Kaplan M, Doudna JA. Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature*. 2014 December 11;516:263-266.
18. Cohen J. How the Battle Lines Over CRISPR Were Drawn [Internet]. AAAS; 2017 February 15 [cited 2017 March 2]. Available from: http://www.sciencemag.org/news/2017/02/how-battle-lines-over-crispr-were-drawn
19. Ran FA et al. In vivo genome editing using Staphylococcus aureus Cas9. *Nature*. 2015 April 9;520(7546):186-91.
20. Finnie I, Williamson C. CRISPR Patent Wars [Internet]. GEN Genetic Engineering & Biotechnology News; 2017 February 6 [cited 2017 March 2]. Available from: http://www.genengnews.com/gen-exclusives/crispr-patent-wars/77900842
21. Wade N. Scientists Seek Moratorium on Edits to Human Genome That Could Be Inherited [Internet]. New York Times; 2015 December 3 [cited 2017 February 28]. Available from: https://www.nytimes.com/2015/12/04/science/crispr-cas9-human-genome-editing-moratorium.html?_r=0
22. Cyranoski D. Chinese scientists to pioneer first human CRISPR trial [Internet]. Nature News; 2016 July 21 [cited 2017 March 2]. Available from: http://www.nature.com/news/chinese-scientists-to-pioneer-first-human-crispr-trial-1.20302
23. Research and Markets. CRISPR and CRISPR-Associated (Cas) Genes Market Analysis By Product (Vector–Based & DNA–Free Cas), By Application (Genome Engineering, Disease Models, Functional Genomics), By End–Use, And Segment Forecasts, 2014-2025 [Internet]. Research and Markets; 2017 Jan [cited 2017 March 8]. Available from: http://www.researchandmarkets.com/research/h987c6/crispr_and
24. Harmon A. Human Gene Editing Receives Science Panel's Support [Internet]. New York Times; 2017 February 14 [cited 2017 March 11]. Available from: https://www.nytimes.com/2017/02/14/health/human-gene-editing-panel.html?_r=0
25. Tang L, Zeng Y, Du Z, Gong M, Peng J, Zhang B et al. CRISRP/Cas9–mediated gene editing in human zygotes using Cas9 protein. *Mol Genet Genomics*. 2017 March 1. Doi: 10.1007/s00438-017-1299-z
26. AAAS. A CRISPR First: Editing Normal Human Embryos [Internet]. AAAS; 2017 March 9 [cited 2017 March 11]. Available from: http://www.sciencemag.org/news/sifter/crispr-first-editing-normal-human-embryos
27. Reyes AP, Lanner F. Towards a CRISPR view of early human development: applications, limitations, and ethical concerns of genome editing in human embryos. *Development*. 2017;144:3-7. Doi: 10.1242/dev.139683