The Nobel Prize in Chemistry 2020: Celebrating the collaborative efforts leading to a tool (CRISPR-Cas) for rewriting a code of life

The Nobel Prize in Chemistry 2020 has been awarded to Emmanuelle Charpentier, from the Max Planck Unit for the Science of Pathogens, Berlin, Germany and Jennifer A. Doudna, from University of California, Berkeley, USA, for the gene-editing technique known as the CRISPR/Cas9 scissors. For the first time in history, the prize has been awarded within nine years of the discovery and the awardees are an all-female team after Marie Curie (1911) and Dorothy Crowfoot Hodgkin (1967), who are single awardees of the Nobel Prize. No doubt, this discovery has opened up new horizons for therapeutic and biotechnological applications.

The story of CRISPR/Cas reiterates how research in basic science opens up doors for innovations! A study on arms race between the tiny bacteria and their tiniest enemy, the viruses (Yes! the bacteria are attacked by viruses, commonly called bacteriophages), has endowed a logical applications.

The Nobel Prize in Chemistry 2020 is being studied in various bacteria found in different habitats including thermal springs, human body, soil, etc. Since the discovery of the simple mechanism of CRISPR-Cas action in Streptococcus pyogenes, this nano-machinery has been beautifully harnessed to edit various genomes, across different kingdoms. The machinery is targeted into the cells with the help of inactivated viruses. Originally known to wade off viruses, the machinery is now used to cure/resist chronic pathogenic viral diseases with the help of viruses. What a smart way!

This year’s Nobel Prize is a celebration of collaborative efforts which paved the way for utilizing the CRISPR-Cas system for genome editing. Let us walk through the journey of Jennifer Anne Doudna and Emmanuelle Charpentier in deciphering a novel mechanism and developing a versatile tool for genome editing.

Jennifer Anne Doudna completed her Ph.D. in 1989 from Harvard Medical School on self-replicating catalytic RNA (that revolutionized RNA research) under the guidance of Jack William Szostak who along with Elizabeth Blackburn and Carol W. Greider won the 2009 Nobel Prize in Physiology or Medicine for the discovery of how chromosomes are protected by telomeres. Her Ph.D. work suggesting the functioning of RNA as polymerase provided an impetus to her future research, a kick start for her interest in RNA biology and its function. Further, to groom her interest she worked as a postdoctoral fellow with Thomas Cech, one of the awardees of the Nobel Prize in Chemistry in 1989, for discovering the catalytic properties of RNA along with Sidney Altman. With him and others, Jennifer crystallized a self-cleaning RNA (ribozyme) discovering its shape and structure. This further provided insights into the RNA’s biological function. In 2000, she received the Alan T. Waterman Award for determining the ribozone structure. She also deciphered the functioning of a small ribozyme in hepatitis D virus and hepatitis B virus. Her discovery this year’s Nobel Prize in Physiology or Medicine was jointly awarded to Harvey Alter, Michael Houghton and Charles Rice; yes, small world for the connections of organisms as well as associated people for them too. Through cryogenic electron microscopy, her team elucidated the way a small RNA element of hepatitis C virus seizes the host’s cellular machinery to make protein for itself. She believed that understanding how RNA could catalyse different chemical reactions would provide insights into the RNA-world hypothesis and open up avenues for therapeutic targets against the RNA viruses. In 2006, Jillian Banfield, a colleague at Berkeley who was interested in understanding the role of CRISPR in RNA interference (a gene silencing by double-stranded RNA) in extremophiles, introduced her to the CRISPR world. The discovery of RNA interference mechanism by Andrew Z. Fire and Craig C. Mello fetched the Nobel Prize in Physiology or Medicine in 2006 (ref. 13).

In 1987, Yoshizumi Ishino and his colleagues serendipitously discovered the partial DNA sequence of CRISPR (was not named as CRISPR then) while sequencing the iap gene in Escherichia coli, a bacteria that resides in the gut. He was one of the first scientists to have detected CRISPRs in E. coli. Francisco Mojica, Alexander Bolotin, Eugene Koonin, and Philippe Horvath, in that order, were the early pioneers to identify and characterize the CRISPR as an adaptive immune system in bacteria. Until 2006, the mechanism of interference was unknown. It was speculated as a putative
RNA-interference based immune system in prokaryotes against the invading viruses. As time progressed, the CRISPR story began to unfold and the intricacies of the interference mechanism were fleshed out. The work by Jennifer’s team provided structural insights into the functional activities of Cas proteins in *Pseudomonas aeruginosa* and *E. coli*17–19. However, the system in these two bacteria is complex with multiple proteins working in concert to execute the function. With such a complex system, the progress in genome editing would have been far slower (had it not been the mechanistic understanding of Cas9). In 2011, on the second day of the ASM conference, Emmanuelle Charpentier proposed a collaboration with Jennifer Doudna after they met in a café in Puerto Rico. She was interested to understand the structural biochemistry of Cas9, furthering the understanding of the CRISPR-mediated mechanism to trim viral DNA20.

Emmanuelle Charpentier has been a highly dynamic person working at nine different institutes in five different countries mainly dissecting out the survival strategies of the pathogens. Her primary focus at the beginning of her career was to do something innovative in medicine. With this aim in mind, she completed her Ph.D. in microbiology from Pasteur Institute in 1995 (refs 20, 21). During her postdoctoral research at Tuomanen’s lab, she worked on mobile genetic elements in *Streptococcus pneumoniae* identifying the mechanism of antimicrobial resistance against vancomycin21. While working on mammalian genes at Pamela Cowin’s lab at New York University, she aspired for developing better genome engineering tools21. In 2002, she started her independent career at University of Vienna understanding how the bacteria modulate their genome and the biochemical pathways22 with special emphasis on RNA- and protein-mediated regulation. Her discovery that small RNA molecules control bacterial pathogenesis23 gained a lot of importance and kick-started her research in RNA-mediated regulation. Later, she got interested in the CRISPR-Cas system of *S. pyogenes* and was delighted to predict and later prove that the trans-acting RNA (tracrRNA) has a role in the functioning of the CRISPR-Cas system24.

She nailed down the working components of type II CRISPR-Cas system, where the CRISPR and tracrRNA work together to form a mature CRISPR-RNA to be targeted against invading viruses25. This was a head start for the CRISPR/Cas9 biology. However, the exact interference mechanism was yet to be understood. Charpentier hypothesized that Cas9, in conjunction with CRISPR and tracrRNA, was required for the dual-RNA-mediated immunity against bacterial viruses25. With this idea in mind, she approached Jennifer Doudna for deducing the structural biochemistry of Cas9. The short and intense collaboration turned out to be highly successful as they deciphered the mechanism of CRISPR action2. The mechanism was simple and precise with as few as three components involved in its action2. It was soon clear to them that the mechanism could be exploited to edit the genetic material. They tried and tested the same in a test-tube on a gene encoding GFP from a jellyfish. The results of these experiments were published in *Science*2. Within no time of this publication, various scientists exploited the machinery for programmable genome engineering. This editing method was simple, precise, less laborious, quick, and less expensive (150 times less expensive) as compared to the programmable nucleases, Zinc Finger Nucleases (ZFN) and Transcription Activator Like Effector Nucleases (TALENs), being used since 1996 and 2010 respectively26,27. ZFNs and TALENs are constructed by fusing the DNA binding domains, zinc finger motif (prevalent in eukaryotes) and TALE (from a plant pathogen *Xanthomonas*) with *Fok1 cleavage domain from a restriction endonuclease28. ZFN can be targeted to cut at a specific DNA sequence but at times results in off-target mutations. Whereas TALENs have low off-target effects but is bulkier than ZFNs24.

The CRISPR-Cas9 mediated genome editing is executed in three simple steps: expression of Cas9, generating single guide RNA (sgRNA-tracrRNA and spacer linked together) having 20 bp complementary to the target sequence, and the protospacer adjacent motif (PAM), essential for the system to work, at the 3’ end of the target29. The sgRNA steers Cas9 to the target site where Cas9 introduces double-stranded break in the DNA20,23. Then, a non-homologous end-joining repair mechanism introduces random insertion/deletions mutating the gene by altering its reading frame. Later, the Cas9 nuclease was modified to introduce a single cut at the target site promoting homology-directed repair that generally replaces the sequence at the target site24,25. Scientist utilized this mechanism efficiently to repair an error in the genetic code. If the replaced sequence is a corrected version of the mutant gene then this could fix the genetic disorders like cancer that results out of a mutant gene. One can even replace the target sequence with completely different gene allowing gene knock-in. Further refinements were made in the system permitting multiple applications like modulating gene expression, inducing epigenetic changes, fluorescent imaging, and base editing25. Here, a chimeric Cas9 comprising catalytically inactive Cas9 fused to effector domains like activator/ repressor (controlling gene expression), epigenetic modifier (inducing epigenetic modification) and green-fluorescent protein (for imaging with fluorescence microscopy)25 is used. Even though Cas9 (from *S. pyogenes*) has been extensively used for various applications, it has certain limitations. Its large size hinders its programming efficiency and delivery to the cells using viral vectors30. Further, its dependence on three nucleotide PAM limits the number of sites that can be targeted5. Moreover, like ZFNs and TALENs it introduces off-target mutations5,25. The Cas9 specificity was improved by developing Cas9 variants like cSpCas9, SpCas9-HF1, etc.23. To overcome the PAM limitation, CRISPR/Cas variants like sp-Cas9-N9, xCas9 have been generated that have either smaller PAM or exhibit PAM flexibility5. Several Cas proteins from other bacteria like CjCas9 (from *Campylobacter jejuni*), Cas12a (Cpf1, from *Francisella novicida*), Cas13 and Cas14 have been exploited for their smaller size5. Of these, Cas13 (ref. 26) and Cas14 (ref. 27) target RNA and single-stranded DNA (ssDNA) respectively5 and can be effectively used to develop resistance against viral infections in plants by RNA26 and ssDNA27 viruses.

The genome programming application is not restricted to cell-lines but is being extended to whole organisms like plants and mice. Rudolf Jaenisch used CRISPR/Cas9 to efficiently perform gene editing in mice25. This method was quite simpler, quicker and cheaper than the previous laborious, lengthier and costlier method using embryonic stem cells, a method that fetched a Nobel Prize in Physiology
or Medicine to Mario Capecchi, Oliver Smithies and Martin Evans in 2007 (ref. 29). The CRISPR/Cas mediated gene editing technique if used wisely and ethically can help curing genetic disorders in humans as well. However, when it comes to manipulation of human genes it becomes a sensitive issue. For some, this means contravening natural selection and is unethical while others opine that, this would help humankind by fixing the faulty genes.

Ironically, this defence system in bacteria can be transformed into an offence, killing its own kind 30–32. It can be used to specifically kill any pathogenic bacteria including the antimicrobial-resistant strains by targeting specific sequences in the bacteria. An exogenous CRISPR/Cas system can be artificially introduced in the bacteria or the bacterial endogenous system can be used to target its own genome 30–32. The applications do not stop here. The system finds utility in diagnostics for COVID-19 as well 33–35 detecting the virus in just 5 minutes 36! The Cas13–RNA complex specifically identifies and binds to a sequence specific to corona-virus followed by the cleavage of the reporter RNA by Cas13, thereby releasing a fluorescent probe. The fluorescent signal is indicative of viral presence.

With so many diverse applications, this system deserves to be called as molecular Swiss-knife rather than molecular scissors.


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