Nobel Prize winner Doudna shares perspectives on future of CRISPR gene editing

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May 12, 2021 -- Small science can translate into big discoveries, according to Nobel Prize laureate Jennifer Doudna, PhD. She discussed how her curiosity in understanding CRISPR led to the gene editing revolution in a talk at the 2021 American Society of Gene & Cell Therapy (ASGCT) virtual meeting.

Doudna was joined by ASGCT President Dr. Stephen Russell, PhD, during a fireside chat at the group's annual meeting on May 11.

Doudna first became interested in CRISPR while working with Jillian Banfield, PhD, a professor at the University of California, Berkeley who was studying bacteria in the environment and how they deal with viruses that are constantly infecting them.

Banfield's research uncovered evidence of a bacterial immune system by acquiring genetic material from bacteriophages (viruses that infect bacteria and archaea) and incorporating it into its own genome as a defense mechanism. This turned out to be CRISPR. In turn, Doudna was intrigued by the idea that bacteria might have figured out a way to use an RNA-guided system for adaptive immunity.

Doudna's work on CRISPR revolved around figuring out how bacteria use CRISPR to recognize specific DNA and RNA and why is it so predominant in bacterial species (roughly 40%) and archaea (nearly 90%). Early efforts allowed her team to figure out that Cas proteins are capable of capturing new sequences and inserting them into the CRISPR system. According to Doudna, this is really what makes it an adaptive pathway.

She explained that RNA-guided systems are dependent on bacterial hosts that are rapidly dividing. Naturally occurring CRISPR systems likely evolve quickly to keep up with the evolution of bacteriophages that are always trying to escape the CRISPR system.

CRISPR systems are highly divergent in nature with many variations on this theme. As the scientific community learns more about these proteins, we can begin to unravel the specific purpose of CRISPR systems.

For instance, recent evidence supports the idea that some bacteriophages carry their own CRISPR to fight "phage wars." In this case, phage have sequences that target other phage which may help them outcompete other phage competitors for survival. Alternatively, there is some evidence suggesting CRISPR can target sequences in the host genome to be employed in gene regulatory pathways rather than genetic defense systems.

Path to the Nobel Prize -- curiosity-based science

As a biochemist at the University of California, Berkeley, Doudna began exploring the CRISPR system. This led to a collaboration with Emmanuelle Charpentier, PhD, to figure out how the guts of the system really worked and how it could be harnessed for a different purpose, namely genome editing.

"CRISPR is a wonderful example of small science," Doudna said. "It's really an example of curiosity-driven research that went in an unexpected direction."

In the process of learning about CRISPR, students in both the Doudna and Charpentier labs conducted the experiment demonstrating that guide RNA (gRNA) could be used to specifically cleave a plasmid. This foundational research was conducted in late 2011 into early 2012.

"In my experience, as a scientist, there are only a handful of experiments that happen in one's career that are extraordinary," Doudna explained.

The CRISPR/Cas9 experiment was one of them for her. But she went on to explain that it was also one of the simplest experiments. Nowadays, most high school students perform similar experiments designed to cut DNA in a test tube using a restriction enzyme and verifying the results.

The experiment conducted by Martin Jinek, PhD, a student in Doudna's lab at the time, had one important difference -- the team didn't know how the Cas9 protein would interact to cut the DNA. The results showed that they could design molecules of RNA that could be combined with Cas9 and used to cut a DNA sequence of their choosing.

Doudna explained that there is a wide range of natural diversity in Cas proteins and that many research teams are pursuing Cas engineering for use in CRISPR systems. She said that she envisions a future where researchers have access to a virtual toolbox which contains Cas proteins with an array of properties to choose from. The toolbox will have the potential to offer new and exciting ways to manipulate DNA and RNA with a variety of applications.

The future of CRISPR

Doudna explained how that one experiment changed her life and the focus of her research. She knew that beyond testing different ways for using CRISPR for genome editing, she wanted to take that work and build on it in ways that will solve real world problems.

This was her motivation for starting the Innovative Genomics Institute, a collaboration between UC Berkeley and UC San Francisco Medical School, in 2013. With additional philanthropic funds, the growing team is focused on applications of CRISPR that make the technology both accessible and affordable to the people who need it.

"It's been a wonderful opportunity to bring together clinicians and scientists who probably wouldn't have any other reason to interact," Doudna noted.

Fast forward, past 2020 when Doudna and Charpentier were awarded the Nobel Prize, to 2021 where there is a whole field of science dedicated to the study of CRISPR. There are two main therapeutic avenues that scientists are currently pursuing: the ex vivo and in vivo application of CRISPR technologies. The pace of scientific discovery in both areas is fast, Doudna said.

For instance, in less than 10 years from the foundational work, patients have already been cured of sickle cell disease using ex vivo CRISPR therapies. In this case, CRISPR is used to edit a patient's stem cells in the lab. After verification, the cells are then replaced back into the patient. This process requires a bone marrow transplant, and Doudna explained that much of the work in this field is focused on reduced costs associated with the treatment and delivery methods that might bypass the need for transplant, and thus reducing costs and making application easier.

With in vivo therapies, the biggest challenge is the delivery challenge, Doudna stated. This is something that many people in the field are currently working to solve. In vivo CRISPR therapeutics must be efficiently and safely delivered to specific cells where they can provide a therapeutic benefit. This is primarily to avoid off-target effects or unwanted genetic alterations.

Doudna believes that this technology is coming soon. The key for in vivo strategies is to deliver the CRISPR system with high efficiency into a cell type of interest, where it is expressed for only a short amount of time. She noted that delivery can be achieved with a number of different approaches, including not only viral vectors but also with nonviral delivery such as messenger RNA (mRNA), nanoparticles, or virus-like particles.

In vivo strategies have had early success in diseases associated with the eye, but Doudna said that now additional targets such as skeletal muscle in muscular dystrophy patients and the brain are being worked on that provide exciting opportunities for the technology.

Doudna and her team remain dedicated to understanding how CRISPR functions. She continues to collaborate with Jillian Banfield's lab, which mines bacterial sequences and passes them to Doudna's lab where they investigate the function of encoded proteins. The team is also exploring opportunities to deliver molecules across cell membranes.

Lastly, Doudna excitedly explained how through a major collaboration she is beginning to work on a new frontier of CRISPR applications in microbes, where the technology is used to edit whole communities of microbes (microbiomes) in situ.

Image caption: Jennifer Doudna, PhD, professor of chemistry and molecular and cell biology at the University of California, Berkeley. Image courtesy of the Royal Society. Licensed under CC BY-SA 4.0.