

CRISPR Pioneer Jennifer Doudna: “Curiosity-Driven, Fundamental Science” Underpins Nobel Prize-Winning Work

Speaking at the inaugural Fred Hutch President’s Seminar, the CRISPR trailblazer outlined goals for gene-editing technology.

October 8, 2024 By Fred Hutch News Service and Sabrina Richards

Nobel Prize winner and CRISPR DNA-editing pioneer [Jennifer A. Doudna, PhD](#), spoke September 19 at Fred Hutch Cancer Center in Seattle. Her lecture kicked off the President’s Seminar series, presented by Fred Hutch President and Director [Thomas J. Lynch, Jr., MD](#). To a packed crowd, Doudna shared the work her lab is doing to improve gene therapies by making CRISPR-based DNA editing technologies more efficient and easier to deliver.

“CRISPR really started with curiosity-driven, fundamental science,” said Doudna, who is a professor at the University of California, Berkeley, and holds the Li Ka Shing Chancellor’s Chair in Biomedical and Health Sciences. She is also a Howard Hughes Medical Institute Investigator and a senior investigator at the Gladstone Institutes.

Doudna and collaborator Emmanuelle Charpentier, PhD, won the [2020 Nobel Prize in Chemistry](#) for their work on the CRISPR-Cas9 (CRISPR for short) bacterial immune system that molecular biologists have co-opted to underpin a revolution in gene editing in biomedicine and agriculture. Through further investigations into the basic nature of Cas9 DNA editing, Doudna and her team hope to make gene therapies cheaper, more widely available and easier on patients.

“Rarely in our careers [as scientists] is there a technique or a technology or a breakthrough that impacts so much of science,” said Lynch, who holds the Raisbeck Endowed Chair.

Doudna grew up in Hilo, Hawaii, and received her undergraduate degree from Pomona College. She earned her PhD from Harvard Medical School studying ribozymes, which are RNA molecules with enzymatic activity. Doudna continued focusing on ribozymes as a postdoctoral fellow and as a faculty member at Yale University. She joined UC Berkeley in 2002.

In 2012, she and Charpentier published their first paper proposing CRISPR as a programmable

genome-editing tool. By December 2023, the Food and Drug Administration had approved Casgevy, the [first gene therapy to use CRISPR-based DNA editing technology](#), to treat [sickle cell disease](#) in people over the age of 12.

Doudna also advocates for policies governing the safe use of CRISPR technology and leads public discussions of the technology's ethical implications. She founded the [Innovative Genomics Institute](#), a collaboration between UC Berkeley, Gladstone and the University of California, San Francisco, dedicated to the development of genome-editing technology. She has also co-founded several biotechnology companies, including Mammoth Biosciences and Intellia Therapeutics.

A technology born from fundamental curiosity

CRISPR grew out of “questions about one of the most basic things that happen in biology, which is that cells get infected with viruses and they have to fight back,” Doudna said.

Certain bacteria have evolved CRISPR-based immune systems to fend off viruses. In bacterial DNA, CRISPR sequences are regions of short palindromic repeats that bookend stretches of viral DNA that the bacterium has swiped from attacking viruses. CRISPR stands for clustered regularly interspaced short palindromic repeats.

Bacteria make RNA copies of a CRISPR sequence and its nearby viral DNA. This RNA “guide” nestles into a CRISPR-associated, or Cas, protein. When viral DNA matches its guide RNA, Cas recognizes that a virus has attacked, and starts chomping. The sliced viral DNA is then degraded and the infection cleared.

Molecular biologists use a lab-designed guide RNA to get Cas to snip any DNA target. Animal and plant cells can repair these DNA breaks, and scientists have taken advantage of this repair process to use CRISPR to insert new DNA sequences. Other DNA editing tools exist, but none are as efficient or as specific as CRISPR.

“In 2007, a colleague of mine at Berkeley introduced me to CRISPR as a possible RNA-guided adaptive immune system,” Doudna said. “I was so fascinated by that possibility that I started my own project to take it in any directions I could.”

What she discovered was that some Cas proteins are good DNA editors — and some are not. Understanding what makes a Cas protein good at editing DNA, what characteristics could improve its editing capabilities, and how to introduce these could provide insights needed to further improve CRISPR-based therapies.

While CRISPR-based gene therapy can correct the mutation causing sickle cell disease, it costs over \$2 million. A patient's blood cells must be removed and edited outside of their body. Then, to ensure that no mutant blood cells remain, patients must undergo a [bone marrow transplant](#) using their edited cells — a brutal process that carries its own risks.

“I want to talk to you about the challenges of making these therapies much more affordable and available to patients,” Doudna said. “Part of [the answer] may come from science and technology. Some of it’s going to come from working with regulators and the FDA. Some of it’s going to come from working with manufacturers and some of it will come from working with patients so they can understand what their options are.”

She focused her presentation on the science and steps her lab members have been taking to address two key questions. First, can Cas9 DNA editing be improved? And how can CRISPR-based gene therapies be delivered inside the body?

“If we had better, faster, more efficient, more accurate editors, we could use less,” which would make CRISPR-based therapies cheaper, she said.

And developing delivery methods that allow the DNA editing to occur inside a patient’s body would, in theory, make the therapies gentler to receive.

Scientific strategies to improve CRISPR-based gene therapy

To better understand what makes a good Cas9 editor, Doudna and her team turned to a bad editor: a Cas9 protein taken from a thermophilic (heat-loving) bacteria, dubbed GeoCas9. While GeoCas9 is more stable at high temperatures and less prone to clumping than SpyCas9, the Cas that started it all, GeoCas9 is dismal at reworking DNA.

Doudna’s team randomly mutated GeoCas9 and then selected the variants that showed improved editing capabilities. They found that mutations that help GeoCas9 make better contact with DNA, maintain its function at magnesium concentrations found in mammalian cells, and relax its preference for a key stretch of DNA just upstream of its target sequence, all improve its ability to edit DNA.

“We think that this is telling us something important about how this type of enzyme is able to latch onto DNA and use the energy of binding to the DNA — that is important for initial recognition of the target sequence — to pry it apart and make it available for downstream chemistry,” Doudna said.

She and her team have also tackled the problem of improving delivery of CRISPR technologies to patients. Right now, the Cas9 protein and its guide RNA are often delivered to cells whose membranes have been made permeable with an electrical field. But this only works for cells removed from the body. Other delivery systems, such as nanoparticles made of lipid membranes, or lentivirus-based particles, could potentially work within the body, but each has its drawbacks.

Viruses can efficiently target very specific cells and make their way to the nucleus where CRISPR can edit DNA, but they’re difficult and expensive to produce. Lipid nanoparticles, in contrast, don’t have the same targeting capabilities, but are “basically shake and bake” as far as manufacturing goes, Doudna said.

Doudna's group is experimenting with what they call enveloped delivery vehicles, or EDVs.

"EDVs are neither a virus nor a lipid nanoparticle — they're something in the middle," she said. "We're really excited about the potential of a particle like this to take the properties that are so useful about viruses ... and combine them with what's so wonderful about lipid nanoparticles."

Researchers in her lab developed [miniEDVs](#), which are 33% smaller by volume than the original EDVs. The team found they could drastically trim the viral protein shell, or capsid, on which the original EDVs were based. They also found that they could enhance DNA editing by using modified Cas9 proteins that included more amino acid sequences that help funnel them to the nucleus.

Wide-ranging audience questions

At the President's Seminar, Doudna fielded questions from audience members, from technical queries related to drug delivery, to sociological concerns about human genetic variation and how it may impact the effectiveness of CRISPR-based gene therapies. She and her collaborators are working to test miniEDV-based CRISPR delivery and editing within the body, and early results are promising, she said.

Doudna also noted that scientists at the Innovative Genomics Institute are working to collect more diverse human genetic data to ensure that gene therapies work in people of any genetic background.

There are many more potential applications for the technology, she said.

"I think the field is really exciting right now," Doudna said. "One of the cool things about these enzymes is that they're really modular and they're really platforms. So it means you can relatively easily engineer them to do other things."

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