THE CRISPR Whisperer

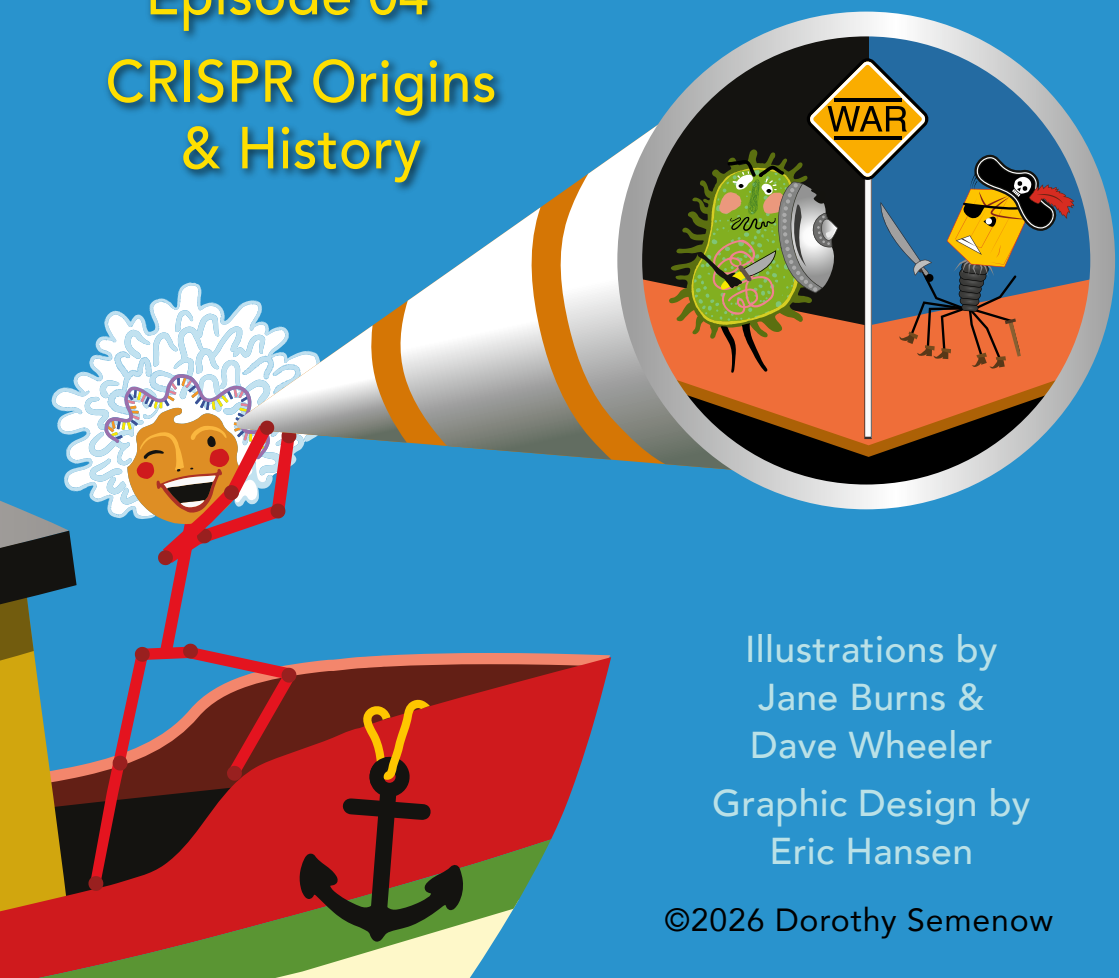
PICTURE Series

for Ages 11 to 111

By Dorothy Semenow, PhD

Episode 04

CRISPR Origins & History



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Episode 04

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Episode 04: CRISPR Origins & History
DEDICATION

To Bacteria, Archaea & Viruses
Without them, human scientists probably wouldn't have CRISPR to edit DNA


You Don't Have To Be a Scientist
To Think Like One!

Art shows. Words tell.
The CRISPR Series is a Science Show and Tell!

The CRISPR Series marries ***Art & Science*** in a humorous, hands-on romp designed to ***spark your creativity*** while clueing you in on an exciting new DNA editing tool called

CRISPR

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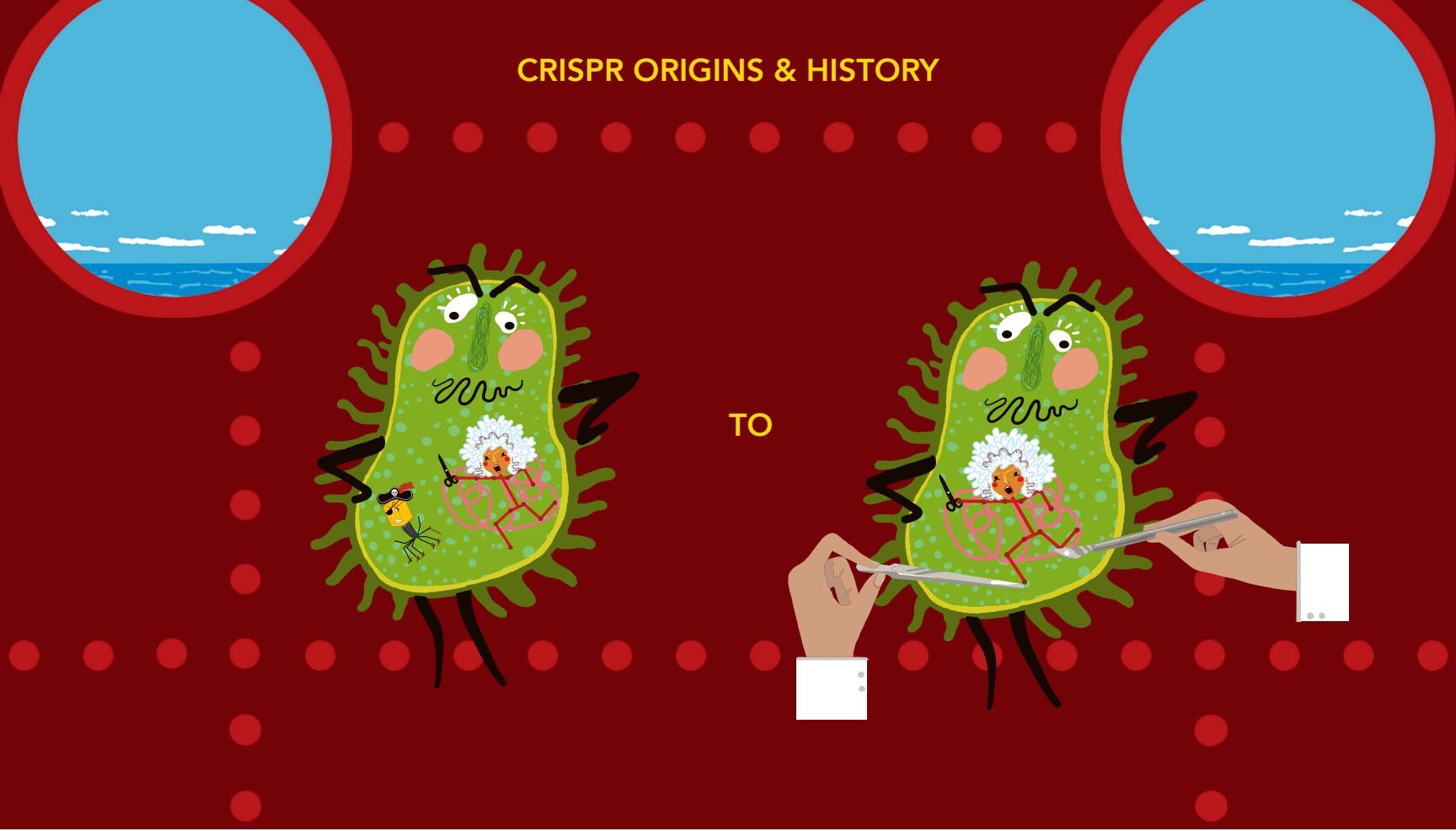
Episode 04: CRISPR Origins & History
TABLE OF CONTENTS

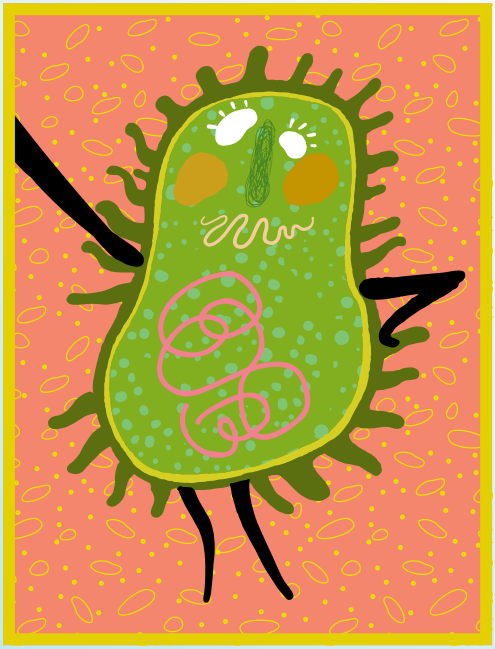


We'll visit the CRISPR Origins & History Exhibit next. But for thousands of years before using CRISPR, humans had been changing plants and animals to serve their purposes. Knowledge of how they did that, covered in Episodes 13 and 14, will broaden your perspective about CRISPR and beyond. Do check it out now or later.

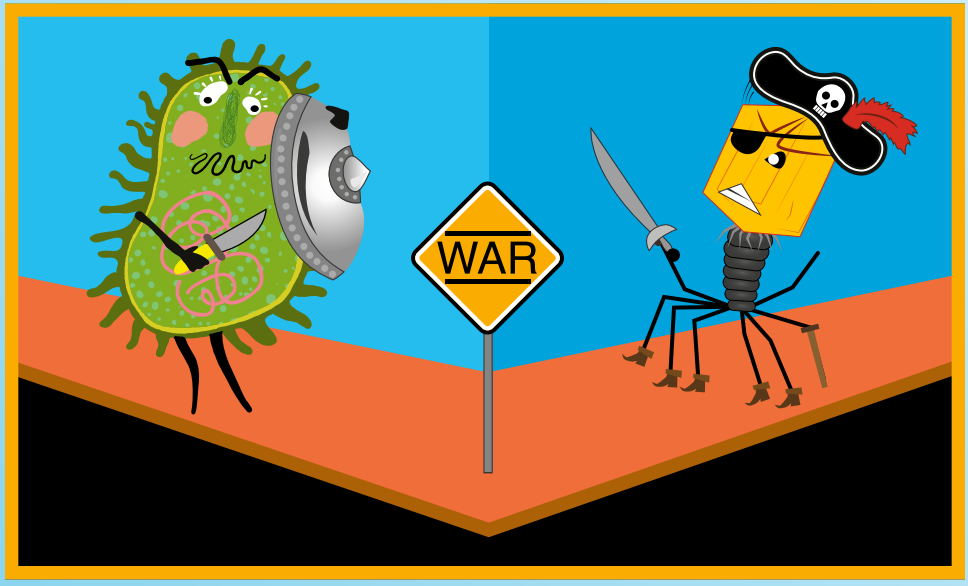
Who Invented CRISPR?

Scientists adapted CRISPR for use in human labs, but they didn't invent her. Bacteria and archaea did that in order to destroy invading viruses. All three are microbes; much more about them ahead.

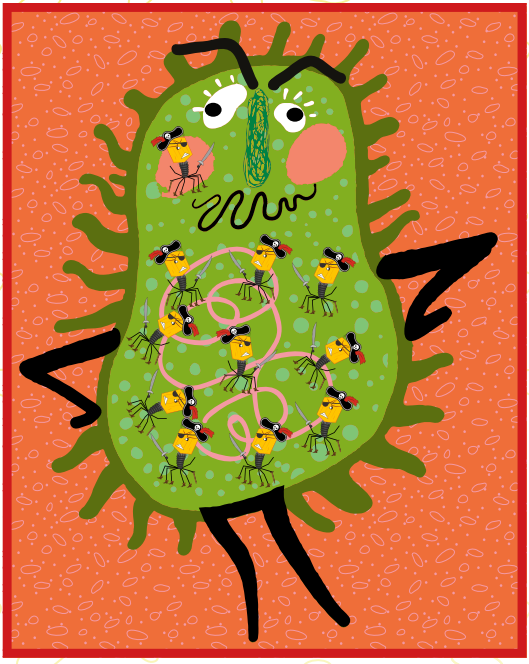
Viruses lack the machinery to copy themselves, so have been invading bacteria and hijacking theirs for a few billion years. For all that time, the two have been at constant war—no surprise they've kept up an ongoing arms race.



Bacteria Selfie



Interminable war is wearing!

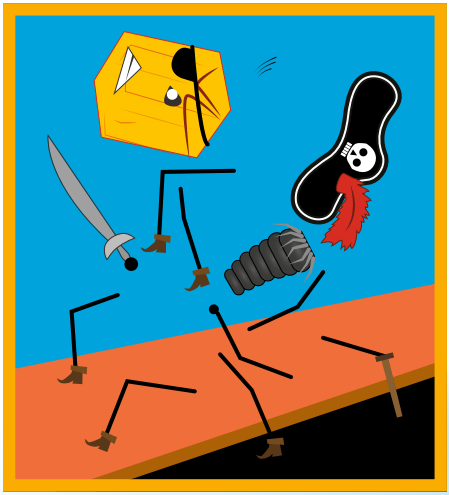


Bacteria: "I'm up in arms and I WILL get even!"

Viruses excel at poking their way though bacteria border walls. Once inside, they commandeer bacterial factories to crank out many copies of themselves. When they run out of space, the viruses blow up their besieged host—then move on to invade, reproduce, and blow up many more.

True to their vow, bacteria do get even. They cache an identifying piece of each virus invader's genome, a mugshot, into their own DNA. Then, when that virus attacks again, the bacteria recognize it and unleash their secret weapon, named CRISPR by Francisco Mojica in 2001 and first published by Ruud Jensen in 2002.

CRISPR:
"I'll defend My Mom to the death!"

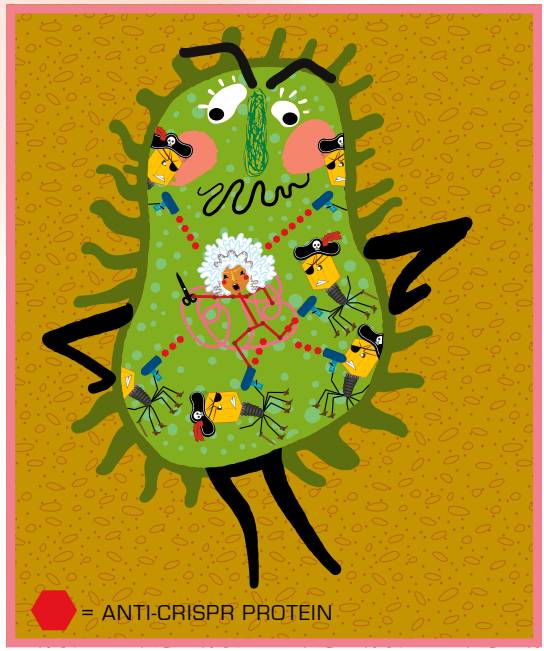


Viruses become cut-ups!

A couple of fast steps later, lickitytrickity splits, CRISPR cuts the reoffending virus to bits. Scientists even award her the lofty status of 'Immune System!'

But, viruses fight back with an anti-CRISPR protein. If enough of them gang up on a host bacterium, they can flood with that new ammo—blunting CRISPR's attack before it kills them.

What's next, a bacterial anti anti-CRISPR or...?



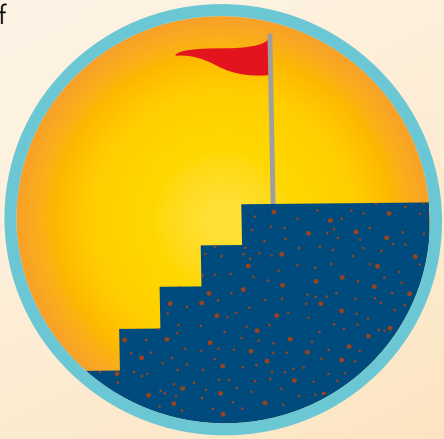
Bacteria: "CURSES! FOILED AGAIN!!"

How Did CRISPR End Up in Human Hands?

Step by Step, You Say? Not One Giant EUREKA?

Filling In the History of CRISPR Breakthrough Discovery

Science progresses step by step, but builders of steps leading up to major discoveries often remain too little recognized. In the case of the CRISPR discovery, science polymath Eric Landers set out to fill this gap with an article in the scientific journal *Cell*, in which he recounted the chain of discoveries leading up to CRISPR's explosive 2012 arrival as the premier genome editor in molecular biology labs.



Lander's fellow scientists generally welcomed his treatment of the important pre-2012 contributions by the relatively unsung "Heroes of CRISPR." But his account of the 2012-2013 CRISPR-as-gene-editor breakthrough, in which he was a stakeholder in the then-ongoing CRISPR patent battle, drew considerable harsh criticism for its slant. Landers had noted his stake when he submitted his article, but the publisher omitted that disclosure. We'll descend into the CRISPR Discovery Mine to delve into that pre-2012 step-by-step odyssey, as so enlighteningly portrayed by Lander.

Might you want to mine the story for insights relevant to challenges in your own life?

First Sighting

The first hint of CRISPR came in 1987, when Japanese molecular biologist Yoshizumi Ishino came upon an unusual repetitive DNA sequence in lab workhorse E. coli bacteria. Most repeats are junk, so Ishino didn't make much of those odd repeats that would later give CRISPR its name.

Mojica Digs In

A few years later, Spanish grad student Francisco Mojica, studying an archaea microbe, came across those same curious DNA repeats, which he described as having multiple copies of a 30-nucleotide base sequence, separated by varied DNA spacers (1993).

Mojica's curiosity kept him mining and minding the repeats-spacers mystery for the next decade.

Mojica on the Edge of Something, but What?

Mojica discovered the structure in distantly as well as closely-related archaea. He searched out Ishino's 1987 report of a similar E. coli structure, which he now realized was present across microbes. He concluded that the DNA repeats and spacers must do something important. Molecular Microbiology published his report in 1995. Mojica kept piling up sightings and, along with workers in other labs, cataloged the mystery structure's key features. CRISPR researcher Rudd Jansen noted the presence nearby of genes for the Cas enzyme, likely related to CRISPR's function. It turned out to be so. Dogged Mojica was hot on the trail!

Mystery Structure About To Be Unmasked and Named

By the late 1990s, Mojica had landed a job on the faculty at the University of Alicante, but lacked funds for further lab work on CRISPR. So he hopped to the computer and shifted to a bioinformatics approach, which revealed 88 spacers similar to known DNA sequences. Two-thirds of those spacer sequences matched sequences from viruses that had invaded the infected microbe. The spacers act somewhat like human antibodies to recognize and destroy repeat offenders. Mojica and Dutch researcher Ruud Jansen first used the CRISPR acronym in print in 2002.

In 2003, Mojica's Aha moment: CRISPR must encode instructions for an adaptive immune system that protects microbes (bacteria and archaea) against future infections by previous virus invaders!

Francisco Mojica found me out!

Realizing that his discovery was important, Mojica immediately wrote up his team's results. BUT WAIT, not so fast! The editors of two leading multidisciplinary science journals, Nature and *Proceedings of the National Academy of Sciences (PNAS), successively rejected Mojica's submission without even sending it out for peer review. The journals' gatekeepers mistakenly judged that the discovery was not new. Next he tried Molecular Microbiology, home of his previous report, but no go there either.*

Increasingly fearful of being scooped, Mojica sent the article to the *Journal of Molecular Evolution*. Finally, after a total of 18 months in the works, it appeared in the February 2005 issue, a dash ahead of two similar reports by Gilles Vergnaud and Alexander Bolotin, published three and six months later, respectively, in other journals.

Publication of all three reports leading up to CRISPR, the 21st century's most momentous biology discovery to date, was delayed due to rejection by powerful editors, who badly misjudged the findings as 'nothing new!'

If it can be that hard to register new evidence-based but unexpected science results, think what that means for funding of riskier science proposals! (More about both those problems later.)

Food Company Zeroing In on CRISPR's crRNA & Cas9 Enzyme

Early in this century, Rhodia Food (Danisco since 2017) tasked Philippe Horvath with fending off phage virus attacks on the bacterial cultures used to produce yogurt and cheese. He learned of CRISPR in 2002; and by 2004, he noticed a clear correlation between CRISPR spacers and phage resistance in his bacteria. He and his colleagues, Rodolphe Barrangou and Sylvain Moineau, found that inserting more CRISPR spacers into genomes of the virus-resistant bacteria increased their resistance. They also found that phages that overcame the resistance had a single base change compared to their mugshot in the bacteria's spacer library (2007)—like crooks who get plastic surgery to disguise their looks!

The small community of CRISPR researchers centered at Rhodia included John van der Oost and Eugene Koonin. Their team created artificial CRISPR arrays, and demonstrated the essential roles of the Cas enzyme and CRISPR RNA (crRNA); they also characterized both of these CRISPR components.

Following the Rhodia team's 2007 confirmation of CRISPR's ability to provide phage resistance, Sylvain Moineau collaborated with that group to show that the Cas9 enzyme cut DNA at a precise position encoded by its target-hunting CRISPR teammate, crRNA. Popular news outlets reported the discovery because it was useful commercially.

Prediction: CRISPR Might Be Used as a Gene Editor!

Then Luciano Marraffini, determined to work on CRISPR, joined the lab of Erik Sontheimer. Together, they demonstrated that CRISPR protected bacteria by identifying and destroying the DNA of virus invaders. ***They were the first to explicitly predict that CRISPR might be repurposed for genome editing in cells other than archaea and bacteria (2008). But they did not test their prediction experimentally, and their US patent application was rejected.***

Here, as is often the case in science, the idea of CRISPR as a highly useful gene editor would likely not have occurred to Marraffini and Sontheimer or other CRISPR investigators had it not been for prior work on other gene editors.

Yang-Gyum Kim, Dana Carroll, and others had pioneered earlier protein gene editors, using proteins called zinc finger nucleases (ZFNs). They relied on two routes used by cells to repair DNA cuts. Non-homologous end-joining (NHEJ) rejoins cut ends, often adding or deleting DNA bases, which shuts down the target gene. Homology-directed repair (HDR), inserts a piece of DNA between the cut ends—just the ticket for gene editing. Next, Adam Bogdanove and Daniel Voytas invented TALENs, proteins like ZNFs, but simpler to design.

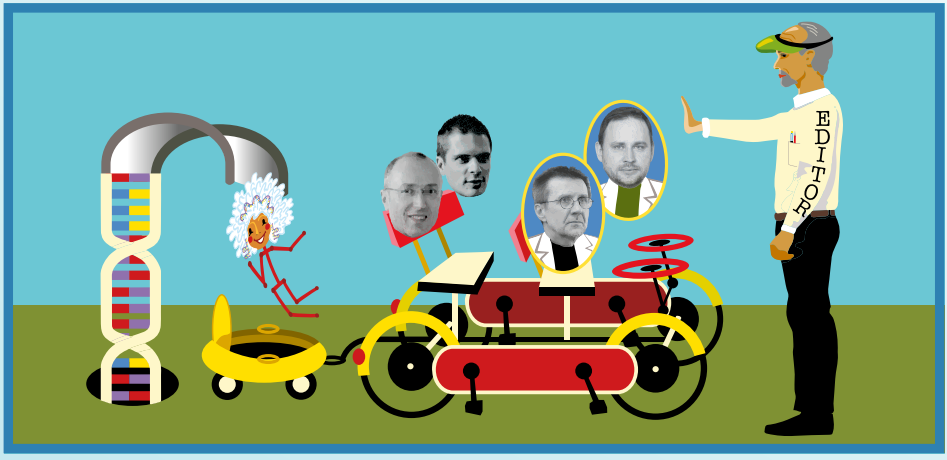
Both laborious procedures, ZFNs and TALENs, were applied to cells from a range of species; they set the stage for better CRISPR editors to come.

Last CRISPR Component Unveiled: Trans-Activating CRISPR RNA (tracrRNA)

Enter onto the CRISPR scene, Emmanuelle Charpentier, later renowned as CRISPR co-developer and co-Nobelist with Jennifer Doudna. She and Jörg Vogel used advanced DNA sequencing to uncover the last CRISPR component, tracrRNA (2011). Without it, CRISPR did not cut DNA!

Crucial Publication Delayed!

The Virginijus Siksnys team hooked up with the yogurt scientists to further explore the CRISPR mechanism, this time in E. coli cells instead of yogurt bacteria. They identified the cutting enzyme as Cas9, pinned down its two active cutting sites, and submitted their report to the journal, Cell. BUT, HERE AGAIN, Lady Luck had her consequential say. The editors at Cell rejected the Siksnys paper in April 2012 without seeking peer review.



The bad luck of the editor draw!

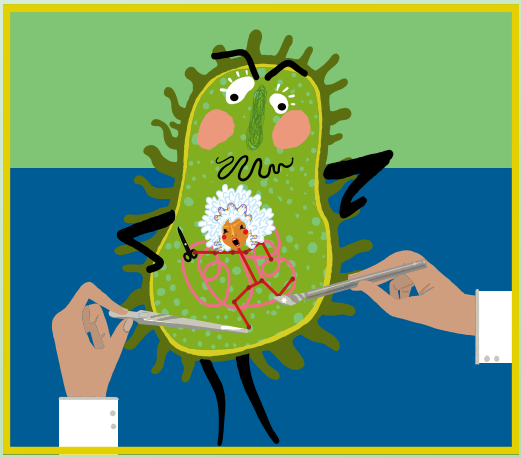
Front Seat: Virginijus Siksnys, Giedrius Gasiunas
Back Seat: Philippe Horvath, Rodolphe Barrangou

The next month, *PNAS* received the Siksny's paper that *Cell* had rejected. After the typical few months for review, it was approved on August 1, 2012.

The BIG CRISPR Editing Breakthrough!

Usually, such a delay in publication of the Siksnys paper wouldn't have mattered. But on June 8, 2012, the editors at *Science* received a similar CRISPR paper from the Jennifer Doudna & Emmanuelle Charpentier team. Recognizing its great importance, the editors fast tracked it to appear in the June 28, 2012 online issue.

Clearly, myriad topnotch hands and minds carried CRISPR from microbes to labs. And, it's true, the race to show that CRISPR works as a gene editor was close.



Scientists say they adopted me, but bacteria decry, "Theft!"

Yet, there's more to the race winners' story than timing of their publication. For while the two teams' papers presented essentially duplicate findings, the Doudna and Charpentier paper also reported an important advance not in the Siksnys paper. They had streamlined the bacterial CRISPR editor by fusing the two essential RNA pieces, crRNA and tracrRNA, into one chimeric segment, named single guide RNA (sgRNA).

Nor was it just luck that advanced Doudna and Charpentier to the prize position in the forward march to claim the breakthrough. Their fearless perseverance to understand CRISPR at the most basic level landed them there.

Ever since Doudna joined Jack Szostak's lab as a Harvard grad student in 1986, she had devoted her scientific career to illuminating the secret life of RNA. She studied RNAs as catalytic enzymes (ribozymes) and learned structural chemistry in order to understand RNA's impressive feats at the molecular level.

It was that RNA expertise that led Jill Banfield, a UC Berkeley professor known for her pioneering research in geomicrobiology and environmental microbiology, to find Doudna via a 2006 web search for a biochemist to help explain what the sequences known as CRISPR-Cas9 do. Banfield introduced Doudna to CRISPR, and Doudna was hooked!

As for Charpentier, when reporting tracrRNA's crucial role in preparing CRISPR's hunter RNA (2011), she had been convinced it did more. She was hellbent on discovering the molecule's additional role/s. "I became obsessed with tracrRNA. I am stubborn. It was important to me to follow up." But that required a biochemist, and neither she nor anyone in her lab filled the bill.

So, at the 2011 Microbiology Conference in Puerto Rico, Charpentier set her sights on biochemist Doudna, who had read Charpentier's CRISPR paper, and jumped on the opportunity to meet her; their collaboration was born.

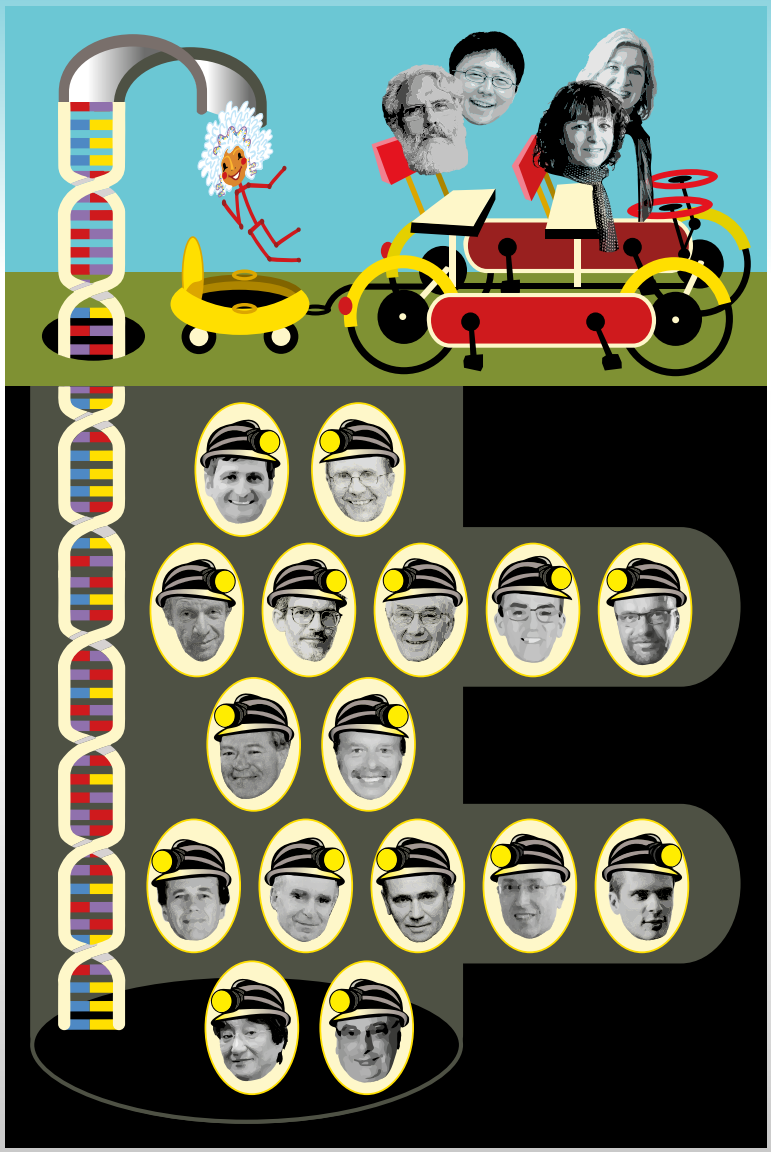
Together, their teams figured out tracrRNA's second role. It acted as a scaffold to position the other CRISPR components to cut the target DNA where specified. Without tracrRNA, the cut did not occur! And, to top it off, Doudna's RNA know-how enabled the teams to fuse the two essential RNAs to form a single guide RNA (sgRNA), an accomplishment that expedited CRISPR's use.

In awarding Doudna and Charpentier the 2020 Nobel Prize in Chemistry, the Royal Swedish Academy of Sciences called CRISPR, "Genetic scissors: a tool for rewriting the code of life"

The press release announcing the Prize stated, "Since Charpentier and Doudna discovered the CRISPR-Cas9 genetic scissors in 2012, their use has exploded. This tool has contributed to many important discoveries in basic research, and plant researchers have been able to develop crops that withstand mold, pests, and drought. In medicine, clinical trials of new cancer therapies are underway, and the dream of being able to cure inherited diseases is about to come true. These genetic scissors have taken the life sciences into a new epoch and, in many ways, are bringing the greatest benefit to humankind." (2020)

PNAS published the Siksnys team's paper on September 4, 2012, without fanfare. Siksnys' contribution didn't make it into media accounts of the discovery. By then, the credit die, with its associated future prizes and billions, had been cast.

Honoring 2020 Nobelists
Jennifer Doudna & Emanuelle Charpentier



It Often Takes Many Diggers & Drivers to Mine a Major Discovery!

Miners

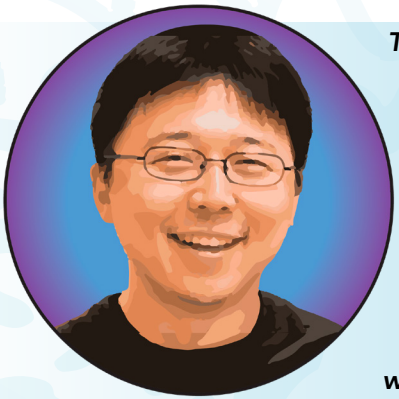
Bottom to Top: Yoshizumi Ishino, Francisco Mojica, Ruud Jansen, Gilles Vergnaud, Alexander Bolotin, Philippe Horvath, Rodolphe Barrangou, Sylvain Moineau, John van der Oost, Eugene Koonin, Luciano Marraffini, Dana Carroll, Adam Bogdanove, Daniel Voytas, Jörg Vogel, Virginijus Siksnys

Cyclists

Front Seat: Emmanuelle Charpentier, Jennifer Doudna

Back Seat: George Church, Feng Zhang

In January 2011, rising gene-editing star Feng Zhang, working as a postdoc in George Church's lab, published the repurposing of the earlier TALEN gene editor to precisely edit genes, and switch them on and off in mammals. A month later, he heard about CRISPR at a talk by microbiologist Michael Gilmore and immediately recognized it as a better method. He was all in!



The Zhang team—Le Cong, F. Ann Ran, the aforementioned Luciano Marraffini (p. 18) and Zhang himself—tinkered with the Doudna & Charpentier team's RNA system to make it cut both DNA strands in mammalian cells. They inserted DNA segments into the CRISPR cuts, using homology-directed repair (HDR, p. 9), and also demonstrated simultaneous multiple site editing within the mammalian genome. The resulting paper was submitted on October 5, 2012 to Science, where it was published online on January 3, 2013.

George Church, at Harvard & Broad Institute, sees the future and is leading us there. He has probably had his hands in more genome research pies than any other science kingpin. No surprise that he, like his former postdoc Zhang, was energetically developing CRISPR cut protocols for use in mammalian cells. Church's team, most notably postdoc bioengineer Prashant Mali, published a paper comparable to Zhang's in the same issue of Science.

The Church lab research targeted embryonic kidney cells, bone marrow leukemia cells, and human *induced pluripotent stem cells*. ***And, like Zhang's, the report included homology-directed repair and simultaneous editing of multiple sites.***

The main difference between the Zhang and Church teams' work was in changes each made to the Doudna & Charpentier CRISPR sgRNA in order to make it work efficiently in mammalian cells. For both teams, this involved RNA fusion to add back some of the RNA sequence deleted by the Doudna-Charpentier team en route to forming their streamlined single guide RNA (sgRNA).

As per the above recounting, the Doudna & Charpentier team and parallel trackers Zhang and Church all staked claims to the CRISPR breakthrough pie.

The account of the CRISPR patent battle ahead tells about that.

Hands-on Breakthrough Heroes Deserve a Front Seat. See also "Unsung Heroes" of CRISPR by Heidi Ledford (*Nature*, July 21, 2016).

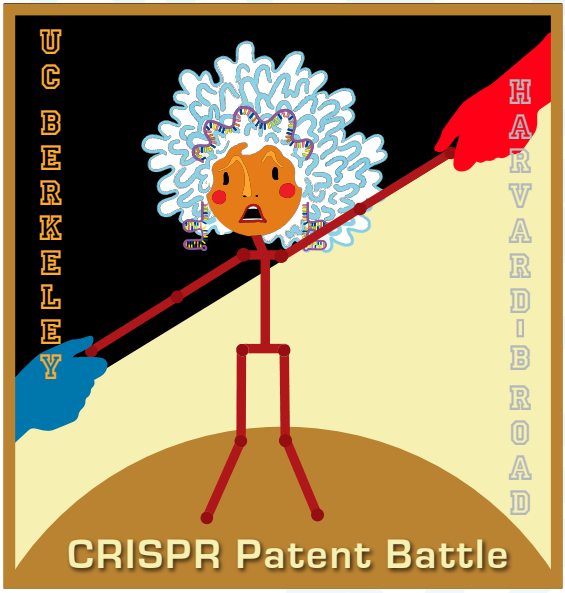
Front Seat (Hands-On Lab Researchers): Prashant Mali, Krzysztof Chylinski, Martin Jinek, Le Chow
Back Seat (Associated Lab Directors): George Church, Emmanuelle Charpentier, Jennifer Doudna, Feng Zhang

Will Battles Never Cease?

If CRISPR thought the battles were over when she moved from bacteria to labs, she was sadly mistaken. No sooner had scientists' commandeered her than she was thrust into a lengthy patent war. The fight was over CRISPR herself! It was the Team West: UC Berkeley, Doudna & Charpentier vs. Team East: Broad Institute/ Harvard & Zhang. Each claimed the right to her patent.

Team West was the first to publish use of CRISPR as a genome editor in *Science* on June 28, 2012. The article reported lab genome editing only in bacteria, but clearly pointed to a broader range of genome targets.

Six months later, Team East reported CRISPR editing of human and mouse cells. In the patent fight that followed, Team East emphasized that Team West's article did not specifically state or implement the method's applicability to eukaryotes (cells with nuclei)—i.e., cells of fungi, plants, and animals including, most notably, humans, but not bacteria.



Stop the madness, I've work to do!

In line with the respective publication dates, Team West applied for the patent first, but Team East paid the $4,000 fee for fast track action and won the patent. Next came Team West's appeals, followed by the patent court's reconsideration of the basis for the decision.

As that billions-dollar dispute has rumbled on, both Teams have amassed robust portfolios of other patents featuring CRISPR. This means that the original patent may lose value over time.

As of mid-2022, Team East gets the patent. We'll leave the combatants to resolve their initial billions-dollar spat, and move on to see why owning rights to CRISPR is so valuable—namely, all the valuable CRISPR uses.

Did We Really Need To Weed Through All Those Credits?

As we've seen, breakthrough discoveries don't arise from one eruption of genius. One discovery breeds another—that's science. All told, there's a lot of credit to be parceled out. Honoring and publicizing pioneers' experiences along the way, is an important part of storytelling.

Perhaps most notably, as hooked as scientists may be on the chase after truth, most care intensely about credit. It is the survival currency of science—reputation, power, money, and love, all rolled into one! That's what the fear of getting scooped is all about.

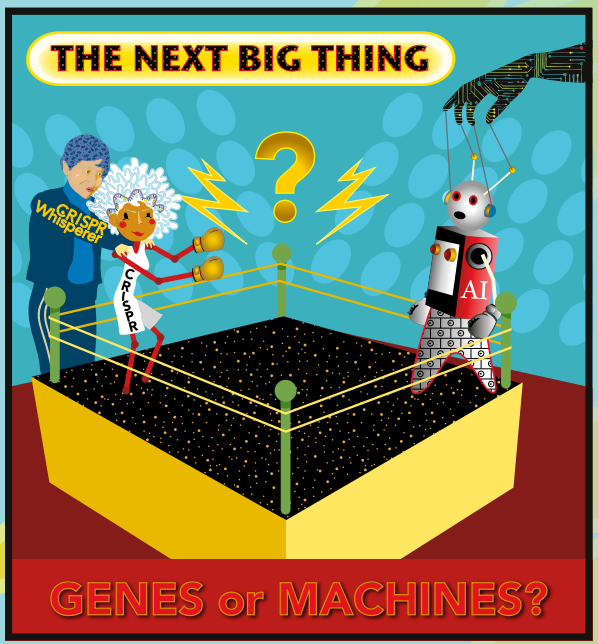
That said, scientists with the strongest drive to chase after truth seem to be best suited for the long haul and least thwarted by obstacles when they occur.

Episode 04 "CRISPR Origins & History
ACKNOWLEDGEMENTS

Eric Landers for his account in "Unsung Heroes" of the pre-2012 CRISPR history, from which this episode is derived

George Church for correction of slant in Lander's "Unsung Heroes" account of 2012-2013 CRISPR-as-gene-editor breakthroughs (private communication)

We close this episode with CRISPR pitching her uniting message—it's your invitation to visit the upcoming episodes of The CRISPR Whisperer Picture Series. We look forward to seeing you there!



Refreshed and recharged, she's set to go—
Next Stop: "CRISPR Plea Letters!"

CRISPR Whispers Picture Series
EPISODES LIST

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and
PGED.org
Personal Genetics Education & Dialogue

Glossary
Episode 04: CRISPR Origins & History

Anti-CRISPR Protein
A group of proteins found in phage bacteria that inhibit the normal activity of CRISPR.

Archaea
Archaea look like bacteria but are actually more closely related to eukaryotes, such as humans. Archaea are single-celled organisms that don't have a nucleus and can only be seen with a microscope. They're found in many different habitats, and many of the first known examples were found in extreme environments.

Bacteria
Microscopic single-celled organisms lacking a distinct nucleus. They inhabit virtually all environments, including soil, water, organic matter, and the bodies of animals.

Bioinformatics
Synthesis of molecular biology and computer science that develops databases and computational tools to store, retrieve, and analyze nucleic acid and protein sequence data.

Cas9 Enzyme
A protein derived from the CRISPR-Cas bacterial immune system that has been co-opted for genome engineering. Uses an RNA molecule as a guide to find a complementary DNA sequence. Once the target DNA is identified, Cas9 cuts both strands. Has been compared to "molecular scissors" or a "genetic scalpel." In CRISPR immunity, cutting viral DNA prevents it from destroying the host cell. In genome engineering, cutting genomic DNA initiates a repair process that ends up making a change or "edit" to its sequence.

crRNA
A protein derived from the CRISPR-Cas bacterial immune system that has been coopted for genome engineering. Uses an RNA molecule as a guide to find a complementary DNA sequence. Once the target DNA is identified, Cas9 cuts both strands. Has been compared to "molecular scissors" or a "genetic scalpel." In CRISPR immunity, cutting viral DNA prevents it from destroying the host cell. In genome engineering, cutting genomic DNA initiates a repair process that ends up making a change or "edit" to its sequence.

DNA Repeats
Patterns of nucleic acids (DNA or RNA) that occur in multiple copies.

DNA Sequence
The process of determining the sequence of nucleotide bases within a DNA molecule.

DNA Spacers
Short nucleic acid sequences (28-36 bp) obtained from previous encounters with viruses or archaea.

Eukaryotes
A domain of organisms whose cells contain a nucleus and other organelles. Eukaryotes are often large and multicellular (e.g. elephants) but can also exist as microscopic, single cells (e.g. yeast). This category of life includes humans. Compare to prokaryotes (bacteria and archaea).

Genome
A full set of chromosomes; all the inheritable traits of an organism.

Genome Editor
Tool to alter the genetic code of a living organism—ZFN, TALEN, or CRISPR. These systems are used to create a double-strand break at a specific DNA site. When the cell repairs the break, the sequence is changed. Can be used to remove, change, or add DNA.

Homology Directed Repair (HDR)
A way for a cell to repair a break in its DNA by "patching" it with a piece of donor DNA. The donor DNA must contain similar sequences, or homology, to the broken DNA ends for it to be incorporated. HDR is a more precise repair pathway than non-homologous end joining. In genome engineering, a researcher designs and adds in the donor DNA, potentially allowing scientists to replace a disease-causing gene with a healthy copy.

Induced Pluripotent Stem Cells (iPSCs)
Cells that have been reprogrammed from mature, adult cells (such as skin or blood cells) into an embryonic-like state. This means that they have the potential to differentiate into any type of cell in the body.

Microbe
A microscopic organism. Can be single-celled or multicellular, and is sometimes used to refer to viruses, although they are not considered to be alive. Examples include bacteria, yeast, and algae.

Non-Homologous End Joining (NHEJ)
A way for a cell to repair a break in its DNA by attaching the free DNA ends. This pathway is "sloppier" than homology-directed repair, and often results in the random addition or removal of nucleotides around the site of the DNA break, causing insertions or deletions in the genetic code. In genome engineering, this allows scientists to stop a gene from working (similar to removing a page from the middle of an instruction manual).

Nucleotide Base Sequence
Order of bases in DNA or RNA.

Phage
Any of a group of viruses that infect specific bacteria.

RNA
Abbreviation of ribonucleic acid. Transcribed from a DNA template and typically used to direct the synthesis of proteins. CRISPR-associated proteins use RNAs as guides to find matching target sequences in DNA.

TALENs (Transcription Activator-Like Effector Nucleases)
A precise genome editor composed of a DNA-binding domain fused to a DNA-cutting domain, used for precise genome editing.

Trans-activating CRISPR RNA (tracrRNA)
The abbreviation tracrRNA for trans-activating CRISPR RNA is pronounced "tracer RNA." In the CRISPR-Cas9 system, the tracrRNA base pairs with the crRNA to form a functional single guide RNA (sgRNA). Cas9 uses the tracrRNA portion of the guide as a handle, while the crRNA spacer sequence directs the complex to a matching viral sequence.

Virus
An infectious entity that can only persist by hijacking a host organism to replicate itself. It has its own genome, but is technically not considered a living organism. Viruses infect all organisms, from humans to plants to microbes. Multicellular organisms have sophisticated immune systems that combat viruses, while CRISPR systems evolved to stop viral infection in bacteria and archaea.

Zinc Finger Nuclease (ZFN)
A genetic engineering tool wherein one portion of the protein recognizes a specific DNA sequence and another part cuts DNA. Made by attaching a series of smaller DNA-binding domains together to recognize a longer DNA sequence. This DNA-binding domain is fused to a nuclease that will cut nearby DNA. Like CRISPR-Cas9 and TALENs, it can be used to alter DNA sequences.