# Special podcast feature: The status and future of CRISPR in agriculture, Part 1

**PNAS:** Welcome to a special feature episode of *Science Sessions*, the podcast of the *Proceedings of the National Academy of Sciences*. I'm Paul Gabrielsen. Imagine a tomato that's spicy like a pepper. Or pig organs that can be compatibly transplanted into humans. Imagine being able to make dozens of edits to an organism's genome in a single step. These are the promises of CRISPR-Cas9, a method of genome editing first proposed in 2012 and adapted from a system that bacteria use to defend against invading viruses. CRISPR proved to be a method that could edit genomes rapidly, precisely, and inexpensively, changing the landscape of genetic engineering and opening new possibilities.

**Urnov:** The impact of being able to change DNA on demand in living cells and thus get a speed and depth of insight that we never dreamt possible to me, frankly, it feels a bit like until CRISPR became a reality, we were a bit like astronomers. We were amazing at cataloging the stars, but touching the stars was laborious. We, as a community of people doing genetic engineering, have a question: Looking at every bit of promise that exists today from CRISPR and other flavors of genetic engineering, what would we like it to do?

**PNAS:** That was Fyodor Urnov, a leading researcher in using CRISPR to treat human disease at the University of California, Berkeley and the scientific cofounder of Tune Therapeutics. Seven years after the original discovery of CRISPR gene editing was reported, in December 2019, the National Academy of Sciences held a colloquium in Irvine, California, to discuss the state of CRISPR.

In this special two-part episode of *Science Sessions*, you'll hear from the colloquium organizers and participants, as well as leaders in the gene editing field, to explore the scientific and regulatory landscape of CRISPR in agriculture. We'll also talk with scientists using CRISPR to modify plants and animals as well as regulatory agencies ensuring the safety and efficacy of United States agricultural products, weighing the risks of gene editing, and deciding how to govern the use of this emerging technology.

The colloquium was titled, "Life 2.0: A CRISPR path to a sustainable planet." Urnov co-organized the colloquium with Barbara Meyer of the University of California, Berkeley and Dana Carroll of the University of Utah.

**Carroll:** I think what we wanted to do was to get a broad view of where the genome editing field stood at the time of the colloquium and get some insight into where it was headed.

**PNAS:** This is Carroll.

**Carroll:** And this included both a wide range of practical uses of genome editing, but also an assessment of where CRISPR and the other technologies stand in society as a whole. Where are those uses going? How are people feeling about it? What are going to be some of the controversies that may arise, based on what's going on in genome editing?

**PNAS:** When CRISPR emerged in 2012, the field of gene editing was already more than a decade old, with technologies such as TALENs and zinc-finger nucleases in development. Carroll was an early pioneer in using zinc-finger nucleases.

**Carroll:** And both of them were really good at making targeted changes in genomic DNA, but they were harder to operate than the CRISPR platform turned out to be. With the zinc-finger nucleases, it was sort of unreliable in design. The TALENs were better at that, but both of them required designing and constructing two new proteins for each target. The platforms were a bit cumbersome in the way you had to operate them.

**PNAS:** CRISPR, which rolls off the tongue, actually stands for Clustered Regularly Interspaced Short Palindromic Repeats. That's scientific lingo for sequences of DNA that work in concert with an enzyme called Cas9 to recognize and cut invading viral DNA. The CRISPR DNA sequences and Cas9 proteins work with such precision that in 2012 a team of researchers wrote that the system could serve as the foundation of "RNA-programmable genome editing." Further experiments in 2013 showed that the method worked and last year Jennifer Doudna and Emmanuelle Charpentier, leaders of the original research team, were awarded the Nobel Prize in Chemistry.

Carroll, who owns stock in Recombinetics, Inc. and receives royalties from Sangamo Therapeutics for their use of zinc-finger nucleases, explains how CRISPR improved on previous methods.

**Carroll:** So, unlike the ZFNs and the TALENs, there's only one protein that's involved with CRISPR and it's the same protein every time. So you only had to design what we call a guide RNA for each new target. And because you just had to make one new, small RNA for each target, you could make a lot of them for many targets simultaneously and multiplex the system in a variety of ways. And this led to very wide adoption of CRISPR for genome editing, even by people who had very little molecular biology experience.

**PNAS:** Beyond applications in medicine, CRISPR is being used in agriculture, which is where the fruits of this technology are more readily realized. It's been used to produce high-yield grains, low-gluten wheat, and disease-resistant rice. Even before the advent of CRISPR as a gene editing technology, food scientists were already harnessing the immune function of CRISPR DNA sequences to improve food products. Here's molecular biologist Rodolphe Barrangou of North Carolina State University. He's a co-founder of five biotechnology companies and a shareholder in five others.

**Barrangou:** I think unbeknownst to most people, already CRISPR has been commercialized in the food space. And as a matter of fact, since 2011, already 10 years in, CRISPR-enhanced phage-resistant natural cultures have been used to ferment milk

into yogurt and cheese across the globe. That means that if you've had or consumed a bite of cheese, a bite of yogurt, a cheeseburger, pizza, a nacho, anywhere in the world in the last 10 years, you've consumed a dairy-fermented product that was enhanced by a CRISPR starter culture.

**PNAS:** Daniel Voytas at the University of Minnesota develops plant genome editing techniques to create crop varieties such as the spicy tomato. He's also the co-inventor of the TALEN technology and cofounder of Calyxt, an agricultural biotechnology company.

**Voytas:** So there are genes in peppers that make the compounds that give peppers their spicy flavor. Those genes exist in tomato. They're just not expressed. They're just not turned on. But we could use gene editing certainly to turn them on to make them expressed. So now our tomatoes would have some of the spicy notes that are characteristic of peppers.

**PNAS:** Gene editing in crops can also achieve a host of other objectives, like producing heart-healthy fats in soybeans. But the process of engineering plant traits takes time.

**Voytas:** So gene editing in plants at present is a very slow process. So we take plant tissue that's in a Petri dish growing sterilely in culture. And then we add our gene editing reagents to some of those cells, we identify which cells have undergone editing. We grow those cells, add hormones to induce those cells to form shoots and roots and ultimately plants. So after about several months to a year, we finally get our gene-edited plant.

**PNAS:** Voytas highlights two recent developments that have accelerated the process. One is the use of developmental regulators. These regulators are protein switches that promote the development of shoots and embryos from edited cells, allowing developers to sidestep the tissue culture step in plant development. Another development, Voytas says, is the use of RNA virus vectors.

**Voytas:** So we've engineered viruses to carry the gene editing reagents. So we can infect a plant grown in an isolated growth chamber with these viruses. And then as the viruses infect the plant, they're carrying the gene editing reagents, [and] infected cells undergo editing. Some of those cells will give rise to flowers and seed. So we can take the seed from this infected plant, and many of those seeds have new gene edits. So in just one life cycle of the plant, we can create hundreds of seeds with hundreds of different gene edits through this process.

**PNAS:** With a streamlined method, developers may be able to engineer a crop such as soybeans to produce palm oil, a desirable food ingredient that imposes environmental costs through deforestation and global shipping—costs that locally grown soybeans producing palm oil wouldn't have. Accelerated gene editing methods bring such innovations into reach.

**Voytas:** When we make a gene edit, we make our best guess. We understand how some plant genes work. And we think if we edit them in certain ways, they'll give us the desirable trait, but sometimes we're wrong, right? Biology is complicated. And so, if it takes a year to make your plant, and then you realize your hypothesis is incorrect, that's a big disappointment. But if you can edit quickly and at scale, then you can undergo the design-test-build cycle much more quickly and you can create prototypes and then, ultimately, the varieties that you want to get out in the field.

**PNAS:** Plants aren't the only field of agriculture touched by gene editing. Researchers are also using CRISPR in livestock as well. Luhan Yang is the cofounder and CEO of Qihan Biotech, a firm employing CRISPR to carry out germline gene editing in pigs—albeit for potential future use in biomedical applications. Germline editing is different from somatic cell editing, in which cells that have already differentiated into their final cell type are edited, such as a skin cell or neuron. In germline editing in animals, an undifferentiated zygote, which later becomes an embryo, is edited. The resulting organism carries the gene edit throughout all of its cells. Yang says there are advantages to germline editing in animals.

**Yang:** As you can imagine, the gene editing approach is not 100% efficient especially when we're treat[ing] a population of cell[s], but using the germline editing, we can get animal[s] carrying homogeneous modification[s] by just doing that once.

For the germline editing, you have the opportunity to isolate [a] single cell, use its proliferation potential to make a copy of it, sample some of them to perform QC, including whole genome sequencing, so that you can select the single cell-derived clone with [a] perfect modification and without off-target. So that even [though] the tool is not perfect, the process can produce [a] perfect cell and animal.

**PNAS:** Yang and her colleagues have demonstrated that, using CRISPR, they can edit dozens of genes in pigs in a single step. The work was published in *Nature Biomedical Engineering* in 2020. The applications of gene-edited pigs go beyond agriculture, as pigs can be used as models to study human diseases and can be engineered to produce high-value bioproducts like hemoglobin, albumin, and blood coagulation factors. The researchers' work also aims to overcome the obstacles to using pig organs for transplantation in humans. One is the potential for rejection of the organ and another is the presence of retroviruses in pigs that could transmit to humans.

**Yang:** Over the last five years what we have done is, first, by using CRISPR to get rid of all the virus activity so that there's no transmission. And on the second part, we demonstrated that we can modify 14 genes in the pig genome including knocking out some major antigen[s] and knocking in human immune inhibitory factors so that we can restore the compatibility of a pig organ with the human immune system.

**PNAS:** George Church of Harvard University and the Massachusetts Institute of Technology is Yang's mentor and collaborator, and a cofounder of Qihan Biotech. He says that the ability of CRISPR to multiplex, or enact multiple edits in the same step, is key to advancing efforts to make pig organs compatible with humans.

**Church:** Well, the idea of xenotransplantation is quite old—at least two decades. But it was, I think, underestimated or at least wise people were waiting until the tools existed. And it was clear that suddenly the tools might exist. And the bucket list for collecting from all the people in the field was on the order of 40 or more changes. We've published 42 changes in pigs at once. A lot of them dedicated to eliminating the endogenous retroviruses, but also all sorts of things having to do with clotting and three different sugar incompatibilities, surfaces, and so on. That, kind of, the collection of everybody's favorite modifications from the field could now be 100% implemented.

**PNAS:** Scientists are still working to increasingly refine the CRISPR technology to minimize so-called off-target mutations, where DNA sequences other than the target sequence are affected. Yang describes how their gene editing process in pigs addresses that concern and other concerns about the safety of gene-edited animals.

**Yang:** We treat a population of pig fibroblasts, isolate single cells into single well[s], let them grow, then sample part of the cell population to do QC, including karyotyping whole-genome sequencing and some long-read sequencing to make sure there is no abnormality in the genome before we perform the somatic cell nuclear transfer to clone a pig embryo and pig. Even [if] the process is perfect, the modification we created in the pig genome may have some unintended biological consequence which can impact the pig['s] health, fertility, and organ function.

**PNAS:** Yang says that it's still early days with Qihan's gene-edited pig populations. So far, she says, they are happy, healthy, and fertile, but more time and more edited individuals are needed to make sure the process is safe.

Thanks for tuning into this special feature episode of *Science Sessions*. In part two of this episode, we will explore safety concerns and the risks of CRISPR gene editing through the eyes of regulatory agencies. You can subscribe to *Science Sessions* on iTunes, Stitcher, Spotify, or wherever you get your podcasts. If you liked this episode, please consider leaving a review and helping us spread the word.