Transgenic Livestock as Drug Factories

By introducing key human genes into mammals, biologists can induce dairy animals to produce therapeutic proteins in their milk

by William H. Velander, Henryk Lubon and William N. Drohan

Exactly one year after her own birth, Genie, our experimental sow, was serenely nursing seven healthy piglets, her milk providing the many nutrients these offspring needed to survive and grow. But unlike other pigs, Genie's milk also contained a substance that some seriously ill people desperately need: human protein C. Traditional methods of obtaining such blood proteins for patients involve processing large quantities of donated human blood or culturing vast numbers of cells in giant stainless-steel reactor vessels. Yet Genie was producing copious amounts of protein C without visible assistance. She was the world's first pig to produce a human protein in her milk.

Genie's ability to manufacture a therapeutic drug in this way was the outcome of a research project conceived almost a decade ago. In collaboration with scientists from the American Red Cross who specialized in providing such blood proteins, we began to consider the possibility of changing the composition of an animal's milk to include some of these critically needed substances. In theory, this approach could generate any required quantity of the various therapeutic blood proteins that are regularly in short supply.

Demand for such drugs comes from many quarters. For instance, hemophiliacs may lack any of several different clotting agents, particularly blood proteins called Factor VIII and Factor IX. Certain people with an inborn deficiency require extra protein C (which acts to control clotting) to supplement their body's meager stores, and patients undergoing joint replacement surgery can benefit from this protein as well. Another important example of the need for therapeutic blood proteins involves people suffering strokes or heart attacks: these cases often demand quick treatment with a protein called tissue plasminogen activator, a substance that can dissolve blood clots. And some people suffering from a debilitating form of emphysema can breathe more easily with infusions of a protein called alpha-1-antitrypsin.

All these proteins are present in donated blood only in tiny amounts, and hence they are currently so difficult to produce that their expense precludes or severely limits their use as drugs. For example, treatment with purified Factor VIII (restricted to those times when someone with hemophilia is actually bleeding) typically costs the patient tens of thousands of dollars every year. The cost of continuous replacement of this blood protein for the same period—a desirable but rarely available option—would exceed $100,000.

Such enormous sums reflect the many problems involved in extracting these proteins from donated blood or establishing specialized production facilities using cultured cells—an enterprise that can require an investment of $25 million or more to supply even modest amounts of a single type of protein. Developing "transgenic" animals such as Genie (that is, creatures that carry genes from other species) demands only a small fraction of such costs. Yet the new breeds simplify procedures enormously and can produce vast quantities of human blood protein. Replacing conventional bioreactors with transgenic livestock thus offers immense economic benefits.

Creating blood proteins in this fashion also stands to better the other cur-

BARRY L. WILLIAMS (*pig*); JOHN HORNER ARCHITECTURAL PHOTOGRAPHY (*bioreactor*)

BIOREACTORS are typically large stainless-steel tanks with complicated controls for maintaining the broth in which countless individual cells are grown. But a new strategy for producing protein-based medicines circumvents the need for such elaborate, and often costly, machinery by using transgenic livestock, such as the pig (*inset*) engineered by the authors to produce one such protein in its milk.

rent practice—purifying them from donated blood—because it would circumvent the risk of contamination with infectious agents. Although blood proteins derived from pooled blood plasma are considered relatively safe now that donors are carefully screened and virus inactivation treatments are routinely applied, the threat from some pathogens always looms. For example, the fear of inadvertently spreading HIV (the AIDS-causing agent) and the hepatitis C virus is spurring researchers to seek substitutes for drugs now derived from human blood. Similarly, recent concerns about Creutzfeldt-Jakob disease (a degenerative disease of the nervous system) has caused some blood products to be withdrawn from the U.S. and Europe. Creating human blood proteins with transgenic livestock that are known to be free of such diseases would deftly sidestep these difficulties.

The many gains that would result from the use of transgenic animals as bioreactors gave us ample reason to pursue our vision of tidy stalls occupied by healthy livestock carrying a few key human genes. But at the outset of our work, we had many worries about the technical hurdles facing us in breeding such transgenic animals and garnering usable quantities of protein from their milk. Fortunately, we were able to progress rapidly, benefiting from a body of trailblazing research that had already been done.

Prior Mousing Around

As early as 1980, Jon W. Gordon and his colleagues at Yale University had determined that a fertilized mouse embryo could incorporate foreign genetic material (DNA) into its chromosomes—the cellular storehouses of genetic material. Shortly afterward, Thomas E. Wagner and his associates at the University of Ohio demonstrated that a gene (a segment of DNA that codes for a particular protein) taken from a rabbit could function in a mouse. Using a finely drawn glass tube of microscopic dimensions, these researchers devised a way to inject a specific fragment of rabbit DNA into a single-cell mouse embryo. Amazingly, that DNA would often become integrated into the mouse's chromosomes, perhaps because it was recognized by the cell as a broken bit of DNA that needed to be repaired.

These researchers then implanted the injected embryos in a surrogate mother mouse and found that some of the mice born to her contained the rabbit gene in all their tissues. These transgenic mice in turn passed the foreign gene on to their offspring in the normal manner, following Mendel's laws of inheritance. The added gene functioned normally in its new host, and these mice made rabbit hemoglobin in their blood.

Another milestone on the road to transgenic animal bioreactors was passed in 1987. Along with their respective colleagues, both Lothar Hennighausen of the National Institute for Kidney and Digestive Diseases and A. John Clark of the Institute of Animal Physiology and Genetics at the Edinburgh Research Station in Scotland established means for activating foreign genes in the mammary glands of mice. Foreign protein molecules created in this way were then secreted directly into a transgenic mouse's milk, where they could be easily collected. These researchers accomplished this feat by combining the foreign gene of interest with a short segment of DNA that normally serves to activate a gene for a mouse milk protein.

Whereas Hennighausen's mice produced the desired human protein (in that case, tissue plasminogen activator) at disappointingly low concentrations, Clark's mice produced 23 grams of a sheep milk protein (known as beta-lactoglobulin) in each liter of milk—approximately matching a mouse's own major milk proteins in abundance. But beta-lactoglobulin was not a human protein in short supply, nor were these tiny mice the proper vehicle to provide useful quantities of milk. So Clark and his colleagues went to work injecting sheep embryos with DNA that contained a medically important human gene.

They used the gene that codes for a blood-clotting factor (Factor IX), along with a segment of sheep DNA that normally switches on the production of beta-lactoglobulin in the mammary gland. Two years later Clark's transgenic sheep secreted Factor IX in their milk—but at barely detectable levels. It was at that juncture that we began our attempts to realize the potential of such pioneering work. But we decided to take a gamble and try a novel strategy.

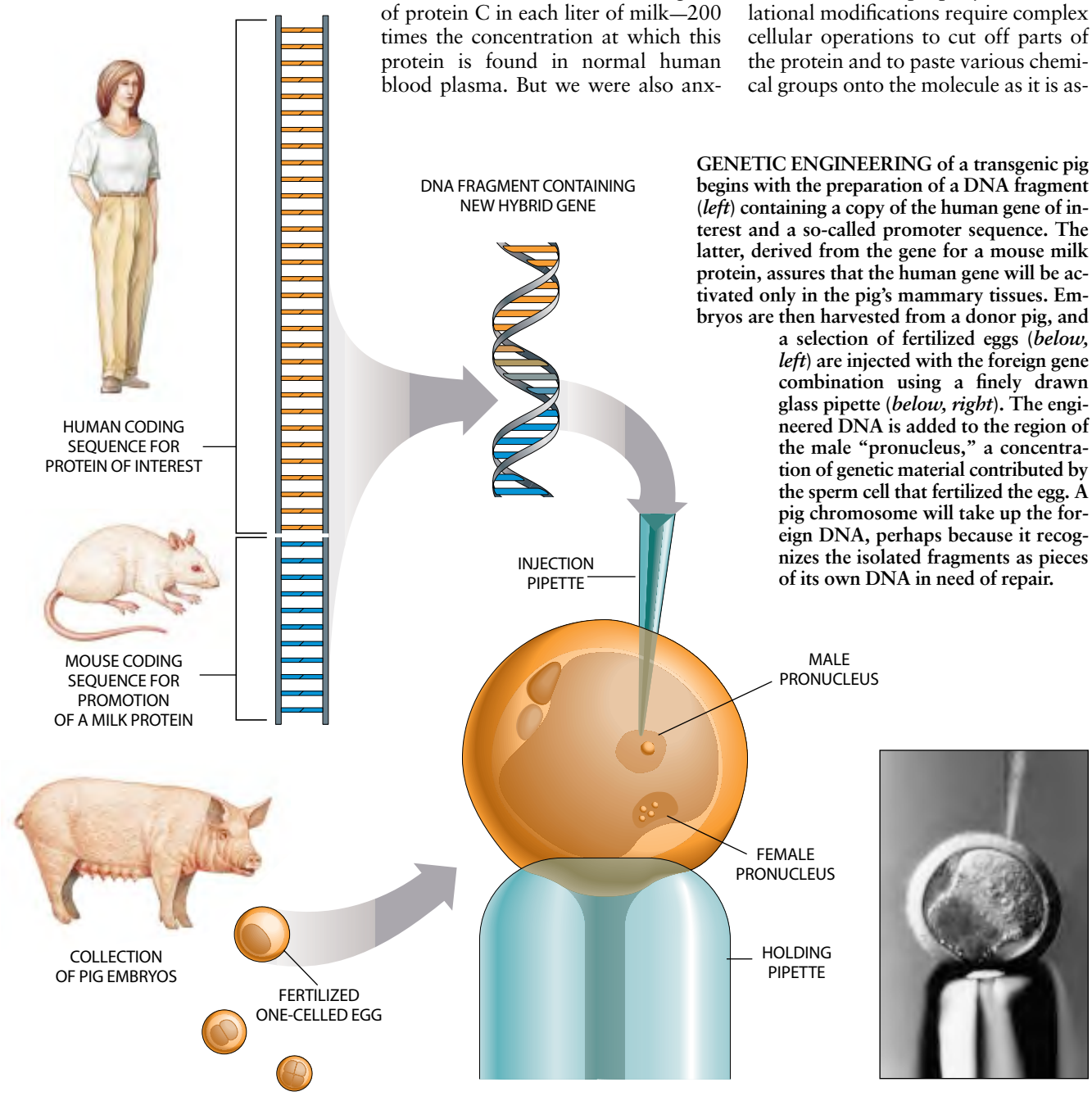
A Pig in a Poke

Whereas other research groups had picked sheep, goats or cows as suitable dairy animals for producing human proteins, we chose to work with pigs instead. Swine offer the advantages of short gestation periods (four months), short generational times (12 months) and large litter sizes (typically 10 to 12 piglets). Thus, producing transgenic pigs is relatively quick compared with transforming other types of livestock. And despite their lack of recognition as dairy animals, pigs do produce quite a lot of milk: a lactating sow generates about 300 liters in a year. The real question for us was whether this unconventional choice of transgenic animal could in fact be made to produce appreciable levels of human protein in its milk.

Toward that end, we decided to use a DNA segment made up of a human gene and the so-called promoter for a major mouse milk protein (called whey acidic protein) that had been characterized by Hennighausen and his colleagues. By injecting this DNA combination into mouse embryos, those researchers were able to augment a mouse's chromosomes so that the creature would produce the desired human protein in its milk. To take advantage of this approach, we, too, fashioned a fragment of DNA that contained the human gene for the target protein (in our case, protein C) and the mouse promoter for whey acidic protein. But we injected this DNA into a set of pig embryos.

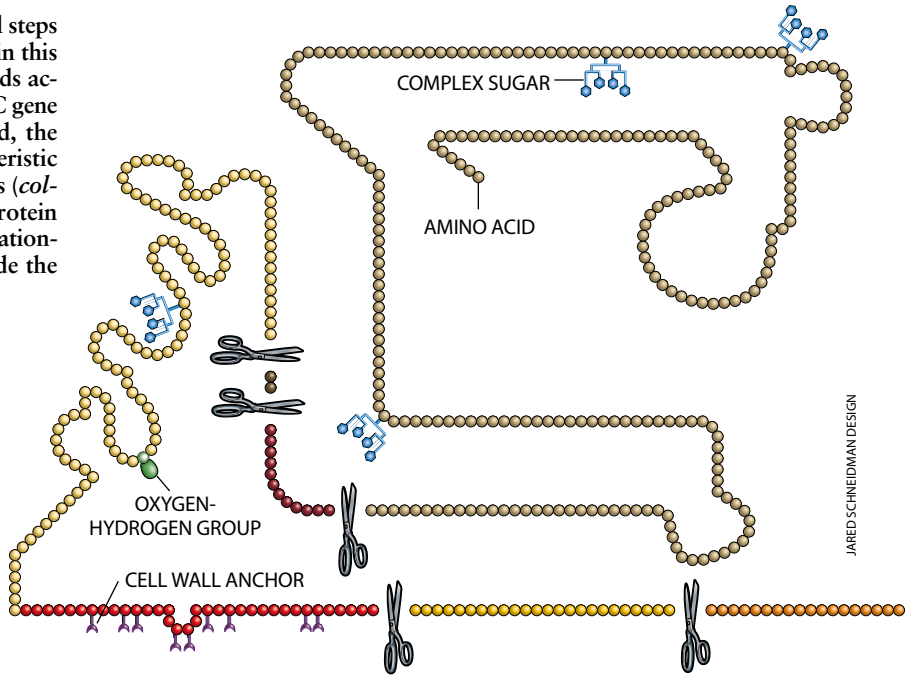
By implanting these fertilized cells in a surrogate mother pig, we could identify—after four months of nervous waiting—a newborn female piglet that carried the foreign DNA in all its cells. But even with this accomplishment, we had to remain patient for another year as our transgenic piglet, Genie, matured. Only then could we find out whether she would indeed produce the human protein in her milk. To our delight, Genie's milk contained protein C. Although the human protein was not as abundant as some of the pig's own milk proteins, it was nonetheless present in substantial amounts, with about one gram of protein C in each liter of milk—200 times the concentration at which this protein is found in normal human blood plasma. But we were also anxious to find out if this pig-made human protein would be biologically active.

We were concerned because the details of protein synthesis inside cells remain somewhat mysterious. The workings of the cellular machinery for reading the genetic code and translating that information into a sequence of amino acids—the building blocks for protein molecules—is, for the most part, well understood by biologists. But there are some subtle manipulations that need to be done by cells after the amino acids are joined together. These so-called post-translational modifications give a newly constructed protein molecule the final shape and chemical composition it needs to function properly. Post-translational modifications require complex cellular operations to cut off parts of the protein and to paste various chemical groups onto the molecule as it is as-



GENETIC ENGINEERING of a transgenic pig begins with the preparation of a DNA fragment (*left*) containing a copy of the human gene of interest and a so-called promoter sequence. The latter, derived from the gene for a mouse milk protein, assures that the human gene will be activated only in the pig's mammary tissues. Embryos are then harvested from a donor pig, and a selection of fertilized eggs (*below, left*) are injected with the foreign gene combination using a finely drawn glass pipette (*below, right*). The engineered DNA is added to the region of the male "pronucleus," a concentration of genetic material contributed by the sperm cell that fertilized the egg. A pig chromosome will take up the foreign DNA, perhaps because it recognizes the isolated fragments as pieces of its own DNA in need of repair.

ROBERTO OSTI (*animals*); JARED SCHNEIDMAN DESIGN (*diagram*); STEPHEN P. BUTLER (*photomicrograph*)



HUMAN PROTEIN C is synthesized in several steps within a cell. The cellular machinery involved in this task starts by stringing together 461 amino acids according to a prescription coded by the protein C gene (a step known as translation). As it is created, the nascent protein molecule folds into a characteristic configuration, forming several distinct domains (*colored regions*). But to function properly, the protein must also undergo several so-called post-translational modifications. These additional steps include the cleaving and removal of certain sections of the protein, as well as the addition of particular chemical groups to specific sites on the amino acid chain.

sembled. Would the cells of Genie's mammary tissue be able to carry out those modifications well enough to make a working version of the human blood protein?

To determine the answer, we had to tackle the new problem of isolating a human blood protein from pig milk. First we removed the milk fat by centrifugation. Then we purified the remaining whey using a procedure that would extract only the biologically active part of the human protein. To our amazement, this component amounted to about one third of the total complement of protein C present. Never before had functional protein C been produced and harvested at such high levels from a transgenic animal—or from a conventional bioreactor. Genie had passed a major test, providing the first practical demonstration that a complex human protein could be produced in the milk of livestock.

Next Year's Model?

We devoted several years to studying Genie and many of her extant offspring and then began to focus our efforts on increasing the concentration of active human protein in the milk. Our intent was to overcome the limitations of mammary tissue in making the needed post-translational modifications. In principle, breaking through those final barriers could triple the output of useful protein molecules produced.

With some painstaking research into the problem, we discovered that most of the protein C remained in an immature, inactive form because there were insufficient amounts of a key processing enzyme named furin—itself a complex protein—within these cells. Hence, we immediately asked ourselves whether we could improve the situation by introducing another foreign gene, one that would allow more of the needed processing enzyme to be made.

To test this possibility quickly, we switched our efforts temporarily from pig to mouse, the fast-breeding mainstay of most transgenic mammal experiments. In 1995 we succeeded in engineering a line of transgenic mice that contained two human genes—one for protein C and one for furin. We arranged for both of these transgenes to switch on in the mammary gland by attaching them to the DNA promoter we had previously incorporated in Genie.

After months of tedious effort in the lab, we were ecstatic to find that these mice were able to secrete the mature form of protein C in their milk. We have thus started development of a new and improved transgenic pig that contains human genes for both protein C and furin. We expect soon to see a pig that produces three times more active protein C than Genie did, and we anticipate that other researchers working with transgenic livestock will also be able to fashion genetic modifications that cause the manufacture of processing enzymes along with the target protein.

Chimerical Visions

The notion of obtaining essentially unlimited quantities of scarce human blood proteins at reasonable cost would have seemed pure fantasy just a short time ago. For more than two decades, molecular biologists and biochemical engineers have labored to overcome the problems of producing even modest amounts of human proteins from large-scale cell culture facilities. Yet making biological pharmaceuticals in huge stainless-steel vats of genetically engineered cells seemed destined to remain an awkward and expensive undertaking.

Such bioreactors are enormously costly to construct, and they prove in operation to be extremely sensitive to small changes in the temperature and composition of the broth in which the cells are grown. In contrast, transgenic livestock bioreactors can be created merely by breeding more animals. Transgenic livestock need only routine attention to control their living conditions and nutrient supply, and yet they can easily produce the desired proteins at much higher concentrations than their metallic counterparts.

Although some risk exists that pathogens could be transmitted from livestock to humans, formal procedures are available to establish pedigreed animals that are free of known diseases. Indeed, such specific-pathogen-free herds are a well-established part of the agriculture industry. In addition, decades of the clinical use of pigs to produce insulin for diabetics give us confidence that swine can readily serve as bioreactors for therapeutic human proteins without presenting undue hazard.

Still, like all new medicines, the human proteins produced in this way need to be carefully tested for safety and effectiveness before the government approves them for widespread use. The

What's Good for Genie…

The advent of transgenic techniques for manipulating livestock also raised legitimate concerns about the health and welfare of the animals altered in this rather unorthodox way. After all, engineered "transgenes" of the kind we implanted in pig embryos can ultimately become part of each and every cell of the mature animals. What if an introduced gene turns on inappropriately and produces the foreign protein in a way that damages the surrounding tissue?

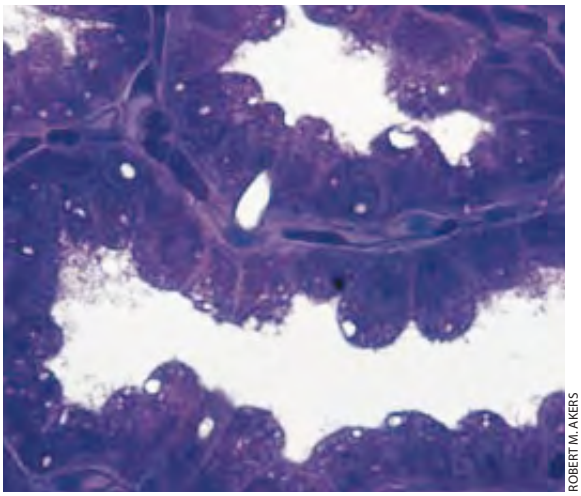
Such worries made it critically important that we design our genetic manipulations so that the foreign gene would be driven into action only in the mammary gland—that is, within tissues that have a natural ability to produce and export protein without harming themselves or their host. We could expect to achieve such targeted control of protein production in our transgenic pigs because we used a promoter from a milk gene—a genetic switch of a type that is present in all mammals.

Yet we recognized that even such well-behaved genes can show some promiscuous activity. The genes we introduced into pigs, for example, also produce small amounts of their foreign proteins in the animals' salivary glands. These tissues are, in fact, quite similar in composition to mammary tissue. So we fully expected this incidental production, and we are quite sure that this minor side effect does not harm the pigs in any way.

The lack of detrimental side effects is crucial—for the animals involved and also for the success of this pioneering method. One of the primary reasons for developing transgenic livestock to supply human proteins is to limit the possibility of transmitting diseases to the recipients of these drugs. Using anything but the healthiest livestock to produce these substances could increase the animals' susceptibility to disease as well as the possibility that they might accidentally pass on some unknown pathogen. Genetically engineering weakened livestock would thus, in the end, only prove self-defeating in the quest to produce safe and plentiful medicines. *—W.H.V.*

first example to be so examined (an anticlotting protein called antithrombin III, manufactured by Genzyme Transgenics Corporation using transgenic goats) began clinical trials just a few months ago.

It is possible that the subtle differences between human and animal cells in the way post-translational modifications are carried out may affect how such proteins function in people. For example, certain modifications cause proteins to be cleared from the blood quickly by the liver, and so we suspect that some of the differences between the animal and human forms of these proteins could actually constitute improvements in the way these substances function as long-lived therapeutic drugs.



ROBERT M. AKERS

MAMMARY TISSUE from a genetically engineered pig contains a dense array of cells (*purple*) that produce a therapeutic human protein. The structure of the mammary gland allows the human protein produced in this way to flow through the secretory channels (*white*), along with other components in the animal's milk.

It is tempting to view the development of transgenic livestock bioreactors purely as a triumph of technology. But the history of this science also highlights the limits of what people can do with sophisticated machines. The mammary gland is optimized to maintain a high density of cells, to deliver to them an ample supply of nutrients and to channel the valuable proteins produced into an easily harvested form. Mammary tissue proves far superior to any cell-culture apparatus ever engineered for these tasks. Despite all their efforts to improve industrial cell-culture facilities, it turns out that a generation of biochemical engineers were unable to match the abilities of a tool for making proteins that nature had already honed. SA

The Authors

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GM salmon: FDA's assessment of environmental risks

BEATRICE DE GEA / LOS ANGELES TIMES

Some salmon steaks. Genetically modified salmon drew closer to FDA approval with publication last week of a long-awaited assessment of environmental effects of the fish.

BY ROSIE MESTEL
December 24, 2012, 3:42 p.m.

Genetically modified salmon moved closer to the market last week with release of draft documents from the Food and Drug Administration that assessed the environmental risks posed by AquAdvantage salmon, which grow faster than regular Atlantic salmon.

The agency found, on a preliminary basis, that the GM fish, produced by AquaBounty Technologies of Massachusetts, posed no significant threat.

Both documents -- an environmental assessment and preliminary "finding of no significant impact," known by the policy wonks as a FONSI -- will be published Dec. 26 in the Federal Register and be available for public comment for 60 days.

The assessment focused on the environmental questions. Food safety came earlier. Back in 2010, the FDA concluded that the salmon "is as safe as food from conventional salmon, and there is a reasonable certainty of no harm from consumption." For example, the flesh of the fish contain no more growth hormone than regular Atlantic salmon, the FDA said -- a concern of opponents to the fish because of the manner in which they were genetically engineered.

The new documents aren't exactly light Yuletide reading, filled as they are with regulatory-agency speak and charts of containment facilities and weather reports from sundry Canadian islands. They go over in some detail the way the fish were created, how and where they will be reared and whether the proposed strategy poses risks to wild salmon or otherwise on the environment.

Here are a few of the points, but anyone who's really interested or concerned should probably wade through the entire document, fortified perhaps with some brandy-spiked eggnog.

How is the salmon genetically modified?

AquAdvantage salmon is Atlantic salmon engineered with a gene from chinook salmon. The gene carries instruction for making growth hormone; that gene is attached to -- and activated by -- a piece of DNA from an ocean pout. The hybrid DNA was microinjected into fertilized salmon eggs back in 1989, to create the first "founder" GM fish. Because of the growth hormone supplied by the added gene, the salmon reaches smolt stage in its lifestyle faster than other Atlantic salmon. (Smolt is the stage when the salmon becomes silvery and would be ready to migrate to the ocean.) Faster growth time to smolt cuts down on feed costs and time to market and thus would make land-based salmon farming more economical, says AquaBounty Technologies, makers of the GM salmon. The FDA notes that 99% of the Atlantic salmon we eat in this country comes from farmed salmon operations in Canada Chile, Norway and Scotland.

How would it be farmed?

Unlike conventionally farmed salmon, the proposal the company put before the FDA doesn't involve farming the fish in net pens in the ocean. Instead, fertilized eggs would be created in inland tanks in Canada (on Prince Edward Island) and the eggs would be transported to an

inland facility in Panama to reach maturity in tanks. The farmed fish would be 100% female and almost all triploid — meaning they carry three copies of every chromosome in each cell instead of the normal two. That makes them sterile. They would be processed in Panama, and salmon fillets and steaks would then be transported to the U.S.

What did the environmental assessment look at?

It reviewed the scientific evidence to draw conclusions on a number of essential questions: the likelihood that the salmon would escape from the facilities, and, if they did escape, how likely they'd be to survive, disperse, reproduce and establish themselves in the wild; and the likely environmental effects within the U.S. if all those things happened. Environmental effects in Canada and Panama are not within the FDA's purview, but the FDA noted that potential physical effects on the U.S. would have to depend on security and containment of the facilities in Panama and Canada, so it looked at those. Those countries also have their own rules and regulations for assessing genetically modified animals.

The FDA wrote that the plans for these salmon confine them physically, geographically and biologically: The fish are sterile, grown in facilities on land with redundant containment measures, and the report goes into detail about all of these. For example, you can read about the confinements starting on Page 13 and later on in more detail, including a table (on Page 46) and figure (Page 47) that describes the various physical containment measures planned at Prince Edward Island; followed by a table and figure for the Panama facility on pages 52 and 53.

The agency concluded that the probability of escape, reproduction and establishment in the wild or harm to the Atlantic salmon or the human environment in the U.S. were very unlikely.

Some opponents of the AquAdvantage salmon expressed concerns, given the distressed state of wild Atlantic salmon fisheries, that the U.S. Fish and Wildlife Service and National Marine Fisheries Service were not involved in the environmental assessment. FDA spokeswoman Shelly Burgess said that both agencies (as well as the U.S. Department of Agriculture) provided comments on the draft. You can read their comments on pages 135 and 136 of the report. And starting on Page 100, you can read about all the various agencies who were consulted during the process.

The Fish and Wildlife Service noted that approval would be only for the planned two facilities on Prince Edward Island and in Panama. And it wrote: "Concern for effects on listed Atlantic salmon would arise if there were a detectable probability that the transgenic salmon could interbreed or compete with or consume the listed fish. Given the nature of the facilities described, any of these outcomes appears to be extremely unlikely, and your 'no effect' determination seems well supported for approval."

But the Fish and Wildlife Service also noted that this was based on the farming scheme as currently laid out. If more facilities were planned, or facilities different in kind were planned, or facilities in the United States planned, AquaBounty would have to apply to the FDA each time and the FDA would review any major or moderate changes in plans. The FDA said in the draft environmental assessment that ocean-based pens were a nonstarter because farmed salmon escape from them.

Would the genetically modified fish carry labels?

The hard plastic coolers transporting fish eggs in transit from Prince Edward Island to Panama would be labeled. But there no decision has been made on labeling of the final product. "Should FDA approve the application related to AquAdvantage Salmon, the agency will make a determination on whether food derived from AquAdvantage Salmon requires additional labeling," Burgess said.

What happens next?

The FDA could decide to move ahead and finalize that FONSI, paving the way toward ultimate approval of the salmon, or it could decide to do a more detailed environmental analysis. (Page 9 provides a figure describing the steps involved approval of a genetically engineered animal.)

In an email, FDA Burgess said: "FDA will review the comments it receives from the public regarding this draft [Environmental Assessment] and preliminary FONSI before making a decision on whether to prepare a final [Environmental Assessment] and FONSI, or to prepare an [Environmental Impact Statement]. In addition, FDA will complete the review of the AquAdvantage Salmon application and will reach a decision on approval. At this point it is not possible to predict a timeline for when these decisions will be made."

Though "environmental assessment" and "environmental impact statement" may sound very much alike, they are not. An environmental assessment is a more concise document that is prepared, in part, to determine whether agencies need to take a more detailed look. Not that these are exactly slim documents: The one prepared for the GM salmon was 145 pages long.

As explained by the EPA, environmental impact statements "are generally prepared for projects that the proposing agency views as having significant prospective environmental impacts." The FDA doesn't see that being the case for the salmon.

CRISPR, THE DISRUPTOR

BY HEIDI LEDFORD

A powerful gene-editing technology is the biggest game changer to hit biology since PCR. But with its huge potential come pressing concerns.

Three years ago, Bruce Conklin came across a method that made him change the course of his lab.

Conklin, a geneticist at the Gladstone Institutes in San Francisco, California, had been trying to work out how variations in DNA affect various human diseases, but his tools were cumbersome. When he worked with cells from patients, it was hard to know which sequences were important for disease and which were just background noise. And engineering a mutation into cells was expensive and laborious work. "It was a student's entire thesis to change one gene," he says.

Then, in 2012, he read about a newly published technique[1] called CRISPR that would allow researchers to quickly change the DNA of nearly any organism — including humans. Soon after, Conklin abandoned his previous approach to modelling disease and adopted this new one. His lab is now feverishly altering genes associated with various heart conditions. "CRISPR is turning everything on its head," he says.

The sentiment is widely shared: CRISPR is causing a major upheaval in biomedical research. Unlike other gene-editing methods, it is cheap, quick and easy to use, and it has swept through labs around the world as a result. Researchers hope to use it to adjust human genes to eliminate diseases, create hardier plants, wipe out pathogens and much more besides. "I've seen two huge developments since I've been in science: CRISPR and PCR," says John Schimenti, a geneticist at Cornell University in Ithaca, New York. Like PCR, the gene-amplification method that revolutionized genetic engineering after its invention in 1985, "CRISPR is impacting the life sciences in so many ways," he says.

But although CRISPR has much to offer, some scientists are worried

ILLUSTRATIONS BY SÉBASTIEN THIBAULT

that the field's breakneck pace leaves little time for addressing the ethical and safety concerns such experiments can raise. The problem was thrust into the spotlight in April, when news broke that scientists had used CRISPR to engineer human embryos (see *Nature* **520,** 593–595; 2015). The embryos they used were unable to result in a live birth, but the report[2] has generated heated debate over whether and how CRISPR should be used to make heritable changes to the human genome. And there are other concerns. Some scientists want to see more studies that probe whether the technique generates stray and potentially risky genome edits; others worry that edited organisms could disrupt entire ecosystems. "This power is so easily accessible by labs — you don't need a very expensive piece of equipment and people don't need to get many years of training to do this," says Stanley Qi, a systems biologist at Stanford University in California. "We should think carefully about how we are going to use that power."

RESEARCH REVOLUTION

Biologists have long been able to edit genomes with molecular tools. About ten years ago, they became excited by enzymes called zinc finger nucleases that promised to do this accurately and efficiently. But zinc fingers, which cost US$5,000 or more to order, were not widely adopted because they are difficult to engineer and expensive, says James Haber, a molecular biologist at Brandeis University in Waltham, Massachusetts. CRISPR works differently: it relies on an enzyme called Cas9 that uses a guide RNA molecule to home in on its target DNA, then edits the DNA to disrupt genes or insert desired sequences. Researchers often need to order only the RNA fragment; the other components can be bought off the shelf. Total cost: as little as $30. "That effectively democratized the technology so that everyone is using it," says Haber. "It's a huge revolution."

CRISPR methodology is quickly eclipsing zinc finger nucleases and other editing tools (see 'The rise of CRISPR'). For some, that means abandoning techniques they had taken years to perfect. "I'm depressed," says Bill Skarnes, a geneticist at the Wellcome Trust Sanger Institute in Hinxton, UK, "but I'm also excited." Skarnes had spent much of his career using a technology introduced in the mid-1980s: inserting DNA into embryonic stem cells and then using those cells to generate genetically modified mice. The technique became a laboratory workhorse, but it was also time-consuming and costly. CRISPR takes a fraction of the time, and Skarnes adopted the technique two years ago.

Researchers have traditionally relied heavily on model organisms such as mice and fruit flies, partly because they were the only species that came with a good tool kit for genetic manipulation. Now CRISPR is making it possible to edit genes in many more organisms. In April, for example, researchers at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, reported using CRISPR to study *Candida albicans*, a fungus that is particularly deadly in people with weakened immune systems, but had been difficult to genetically manipulate in the lab[3]. Jennifer Doudna, a CRISPR pioneer at the University of California, Berkeley, is keeping a list of CRISPR-altered creatures. So far, she has three dozen entries, including disease-causing parasites called trypanosomes and yeasts used to make biofuels.

Yet the rapid progress has its drawbacks. "People just don't have the time to characterize some of the very basic parameters of the system," says Bo Huang, a biophysicist at the University of California, San Francisco. "There is a mentality that as long as it works, we don't have to understand how or why it works." That means that researchers occasionally run up against glitches. Huang and his lab struggled for two months to adapt CRISPR for use in imaging studies. He suspects that the delay would have been shorter had more been known about how to optimize the design of guide RNAs, a basic but important nuance.

By and large, researchers see these gaps as a minor price to pay for a powerful technique. But Doudna has begun to have more serious concerns about safety. Her worries began at a meeting in 2014, when she saw a postdoc present work in which a virus was engineered to carry the CRISPR components into mice. The mice breathed in the virus, allowing the CRISPR system to engineer mutations and create a model for human lung cancer[4]. Doudna got a chill; a minor mistake in the design of the guide RNA could result in a CRISPR that worked in human lungs as well. "It seemed incredibly scary that you might have students who were working with such a thing," she says. "It's important for people to appreciate what this technology can do."

> "There is a mentality that as long as it works, we don't have to understand how or why it works."

Andrea Ventura, a cancer researcher at Memorial Sloan Kettering Cancer Center in New York and a lead author of the work, says that his lab carefully considered the safety implications: the guide sequences were designed to target genome regions that were unique to mice, and the virus was disabled such that it could not replicate. He agrees that it is important to anticipate even remote risks. "The guides are not designed to cut the human genome, but you never know," he says. "It's not very likely, but it still needs to be considered."

EDITING OUT DISEASE

Last year, bioengineer Daniel Anderson of the Massachusetts Institute of Technology in Cambridge and his colleagues used CRISPR in mice to correct a mutation associated with a human metabolic disease called tyrosinaemia[5]. It was the first use of CRISPR to fix a disease-causing mutation in an adult animal — and an important step towards using the technology for gene therapy in humans.

The idea that CRISPR could accelerate the gene-therapy field is a major source of excitement in scientific and biotechnology circles. But as well as highlighting the potential, Anderson's study showed how far there is to go. To deliver the Cas9 enzyme and its guide RNA into the target organ, the liver, the team had to pump large volumes of liquid into blood vessels — something that is not generally considered feasible in people. And the experiments corrected the disease-causing mutation in just 0.4% of the cells, which is not enough to have an impact on many diseases.

Over the past two years, a handful of companies have sprung up to develop CRISPR-based gene therapy, and Anderson and others say that the first clinical trials of such a treatment could happen in the next one or two years. Those first trials will probably be scenarios in which the CRISPR components can be injected directly into tissues, such as those in the eye, or in which cells can be removed from the body, engineered in the lab and then put back. For example, blood-forming stem cells might be corrected to treat conditions such as sickle-cell disease or β-thalassaemia. It will be a bigger challenge to deliver the enzyme and guide RNA into many other tissues, but researchers hope that the technique could one day be used to tackle a wider range of genetic diseases.

Yet many scientists caution that there is much to do before CRISPR can be deployed safely and efficiently. Scientists need to increase the efficiency of editing, but at the same time make sure that they do not introduce changes elsewhere in the genome that have consequences for health. "These enzymes will cut in places other than the places you have designed them to cut, and that has lots of implications," says Haber. "If

"It will be hard to detect whether something has been mutated conventionally or genetically engineered."

you're going to replace somebody's sickle-cell gene in a stem cell, you're going to be asked, 'Well, what other damage might you have done at other sites in the genome?'"

Keith Joung, who studies gene editing at Massachusetts General Hospital in Boston, has been developing methods to hunt down Cas9's off-target cuts. He says that the frequency of such cuts varies widely from cell to cell and from one sequence to another: his lab and others have seen off-target sites with mutation frequencies ranging from 0.1% to more than 60%. Even low-frequency events could potentially be dangerous if they accelerate a cell's growth and lead to cancer, he says.

With so many unanswered questions, it is important to keep expectations of CRISPR under control, says Katrine Bosley, chief executive of Editas, a company in Cambridge, Massachusetts, that is pursuing CRISPR-mediated gene therapy. Bosley is a veteran of commercializing new technologies, and says that usually the hard part is convincing others that an approach will work. "With CRISPR it's almost the opposite," she says. "There's so much excitement and support, but we have to be realistic about what it takes to get there."

CRISPR ON THE FARM

While Anderson and others are aiming to modify DNA in human cells, others are targeting crops and livestock. Before the arrival of gene-editing techniques, this was generally done by inserting a gene into the genome at random positions, along with sequences from bacteria, viruses or other species that drive expression of the gene. But the process is inefficient, and it has always been fodder for critics who dislike the mixing of DNA from different species or worry that the insertion could interrupt other genes. What is more, getting genetically modified crops approved for use is so complex and expensive that most of those that have been modified are large commodity crops such as maize (corn) and soya beans.

With CRISPR, the situation could change: the ease and low cost may make genome editing a viable option for smaller, speciality crops, as well as animals. In the past few years, researchers have used the method to engineer petite pigs and to make disease-resistant wheat and rice. They have also made progress towards engineering dehorned cattle, disease-resistant goats and vitamin-enriched sweet oranges. Doudna anticipates that her list of CRISPR-modified organisms will grow. "There's an interesting opportunity to consider doing experiments or engineering pathways in plants that are not as important commercially but are very interesting from a research perspective — or for home vegetable gardens," she says.

CRISPR's ability to precisely edit existing DNA sequences makes for more-accurate modifications, but it also makes it more difficult for regulators and farmers to identify a modified organism once it has been released. "With gene editing, there's no longer the ability to really track engineered products," says Jennifer Kuzma, who studies science policy at North Carolina State University in Raleigh. "It will be hard to detect whether something has been mutated conventionally or genetically engineered."

That rings alarm bells for opponents of genetically modified crops, and it poses difficult questions for countries trying to work out how to regulate gene-edited plants and animals. In the United States, the Food and Drug Administration has yet to approve any genetically modified animal for human consumption, and it has not yet announced how it will handle gene-edited animals.

Under existing rules, not all crops made by genome editing would require regulation by the US Department of Agriculture (see *Nature* **500,** 389–390; 2013). But in May, the agriculture department began to seek input on how it can improve regulation of genetically modified crops — a move that many have taken as a sign that the agency is re-evaluating its rules in light of technologies such as CRISPR. "The window has been cracked," says Kuzma. "What goes through the window remains to be seen. But the fact that it's even been cracked is pretty exciting."

ENGINEERED ECOSYSTEMS

Beyond the farm, researchers are considering how CRISPR could or should be deployed on organisms in the wild. Much of the attention has focused on a method called gene drive, which can quickly sweep an edited gene through a population. The work is at an early stage, but such a technique could be used to wipe out disease-carrying mosquitoes or ticks, eliminate invasive plants or eradicate herbicide resistance in pigweed, which plagues some US farmers.

Usually, a genetic change in one organism takes a long time to spread through a population. That is because a mutation carried on one of a pair of chromosomes is inherited by only half the offspring. But a gene drive allows a mutation made by CRISPR on one chromosome to copy itself to its partner in every generation, so that nearly all offspring will inherit the change. This means that it will speed through a population exponentially faster than normal (see 'Gene drive') — a mutation engineered into a mosquito could spread through a large population within a season. If that mutation reduced the number of offspring a mosquito produced, then the population could be wiped out, along with any malaria parasites it is carrying.

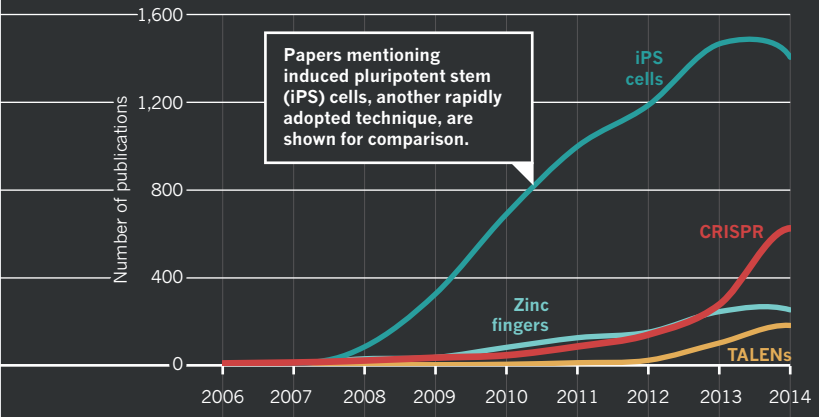
But many researchers are deeply worried that altering an entire population, or eliminating it altogether, could have drastic and unknown consequences for an ecosystem: it might mean that other pests emerge, for example, or it could affect predators higher up the food chain. And ▶

THE RISE OF CRISPR

DNA sequences called CRISPRs (clustered regularly interspaced short palindromic repeats) are part of a bacterial defence system. After researchers showed in 2012 that CRISPRs could be used to edit genomes, use of the tools quickly spread, as reflected by sharp rises in publications, patent applications and funding.

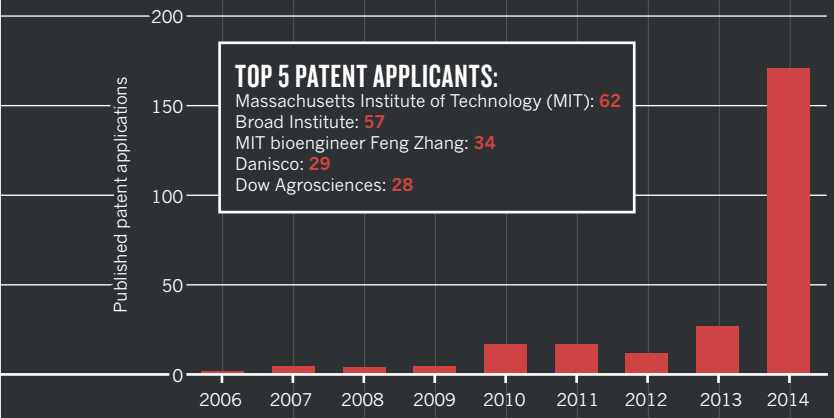
PUBLICATIONS

The number of papers about CRISPR has outstripped the numbers mentioning the gene-editing technologies known as TALENs and zinc fingers.



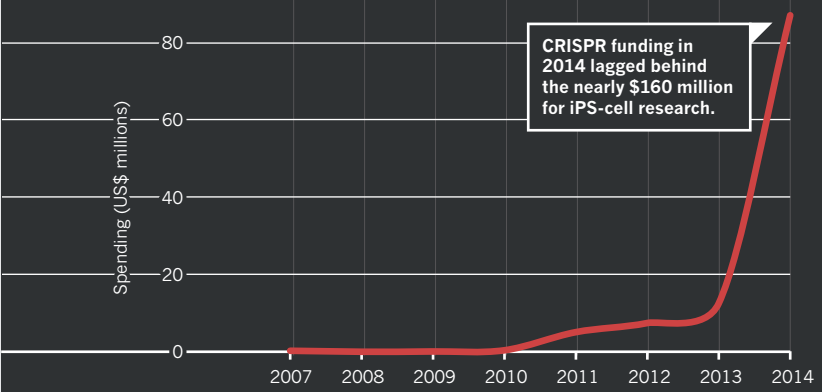
PATENTS

In 2014, worldwide patent applications that mention CRISPR leapt and a patent battle intensified.



FUNDING

A sharp jump in US National Institutes of Health funding for projects involving CRISPR is a harbinger of future advances.



A BRIEF HISTORY OF CRISPR

Key events in the CRISPR story.

December 1987
Researchers find CRISPR sequences in *Escherichia coli*, but do not characterize their function[8].

July 1995
CRISPR sequences are found to be common in other microbes[9].

A cluster of biotech companies has sprung up to use CRISPR technology.

March 2007
Scientists at food company Danisco determine that the repeats are part of a bacterial defence against viruses[10].

October 2011
CARIBOU BIOSCIENCES
Berkeley, California
Focus: Research, industry, therapeutics, agriculture
Raised: $11 MILLION

June 2012
Researchers report that CRISPR can be used to perform genome editing[1].

November 2013
EDITAS MEDICINE
Cambridge, Massachusetts
Focus: Therapeutics
Raised: $43 MILLION

January 2013
CRISPR is used in mouse and human cells, fuelling rapid uptake of the technique by researchers[11–13].

March 2013
The University of California and others file for a patent on the findings[1].

November 2013
CRISPR THERAPEUTICS
Basel, Switzerland
Focus: Therapeutics
Raised: $89 MILLION

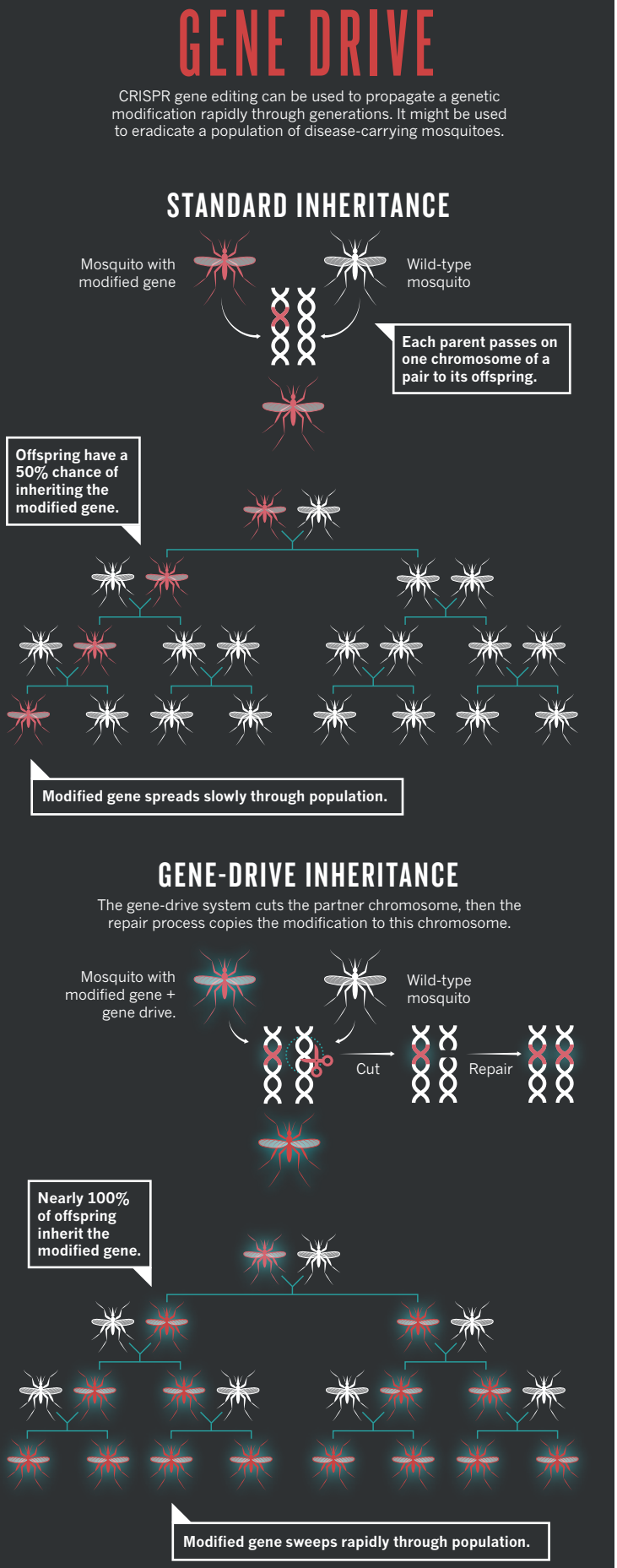
April 2014
MIT and the Broad Institute are granted a patent on CRISPR gene editing, sparking a fierce patent battle.

November 2014
INTELLIA THERAPEUTICS
Cambridge, MA
Focus: Therapeutics
Raised: $15 MILLION

March 2015
Report of the first CRISPR gene drive, which can spread an edited gene rapidly through a population[6].

April 2015
Researchers report that they have edited human embryos with CRISPR, triggering an ethical debate[2].

DESIGN BY **WES FERNANDES**;
SOURCES: PUBLICATIONS: SCOPUS; PATENTS: THE LENS; FUNDING: NIH REPORTER.



researchers are also mindful that a guide RNA could mutate over time such that it targets a different part of the genome. This mutation could then race through the population, with unpredictable effects.

"It has to have a fairly high pay-off, because it has a risk of irreversibility — and unintended or hard-to-calculate consequences for other species," says George Church, a bioengineer at Harvard Medical School in Boston. In April 2014, Church and a team of scientists and policy experts wrote a commentary in *Science*[6] warning researchers about the risks and proposing ways to guard against accidental release of experimental gene drives.

At the time, gene drives seemed a distant prospect. But less than a year later, developmental biologist Ethan Bier of the University of California, San Diego, and his student Valentino Gantz reported that they had designed just such a system in fruit flies[7]. Bier and Gantz had used three layers of boxes to contain their flies and adopted lab safety measures usually used for malaria-carrying mosquitoes. But they did not follow all the guidelines urged by the authors of the commentary, such as devising a method to reverse the engineered change. Bier says that they were conducting their first proof-of-principle experiments, and wanted to know whether the system worked at all before they made it more complex.

For Church and others, this was a clear warning that the democratization of genome editing through CRISPR could have unexpected and undesirable outcomes. "It is essential that national regulatory authorities and international organizations get on top of this — really get on top of it," says Kenneth Oye, a political scientist at the Massachusetts Institute of Technology and lead author of the *Science* commentary. "We need more action." The US National Research Council has formed a panel to discuss gene drives, and other high-level discussions are starting to take place. But Oye is concerned that the science is moving at lightning speed, and that regulatory changes may happen only after a high-profile gene-drive release.

The issue is not black and white. Micky Eubanks, an insect ecologist at Texas A&M University in College Station, says that the idea of gene drives shocked him at first. "My initial gut reaction was 'Oh my god, this is terrible. It's so scary'," he says. "But when you give it more thought and weigh it against the environmental changes that we have already made and continue to make, it would be a drop in the ocean."

Some researchers see lessons for CRISPR in the arc of other new technologies that prompted great excitement, concern and then disappointment when teething troubles hit. Medical geneticist James Wilson of the University of Pennsylvania in Philadelphia was at the centre of booming enthusiasm over gene therapy in the 1990s — only to witness its downfall when a clinical trial went wrong and killed a young man. The field went into a tailspin and has only recently begun to recover. The CRISPR field is still young, Wilson says, and it could be years before its potential is realized. "It's in the exploration stage. These ideas need to ferment."

Then again, Wilson has been bitten by the CRISPR bug. He says that he was sceptical of all the promises being made about it until his own lab began to play with the technique. "It's ultimately going to have a role in human therapeutics," he says. "It's just really spectacular." ■

Heidi Ledford *is a senior reporter for* Nature *in Cambridge, Massachusetts.*

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U.S.

Open Season Is Seen in Gene Editing of Animals

By **AMY HARMON** NOV. 26, 2015

SIOUX CENTER, Iowa — Other than the few small luxuries afforded them, like private access to a large patch of grass, there was nothing to mark the two hornless dairy calves born last spring at a breeding facility here as early specimens in a new era of humanity's dominion over nature.

But unlike a vast majority of their dairy brethren, these calves, both bulls, will never sprout horns. That means they will not need to undergo dehorning, routinely performed by farmers to prevent injuries and a procedure that the American Veterinary Medical Association says is "considered to be quite painful."

Instead, when the calves were both just a single cell in a petri dish, scientists at a start-up company called Recombinetics used the headline-grabbing new tools of gene editing to swap out the smidgen of genetic code that makes dairy cattle have horns for the one that makes Angus beef cattle have none. And the tweak, copied into all of their cells through the normal machinery of DNA replication, will also be passed on to subsequent generations.

"It's pretty cool," said Micah Schouten, the calves' caretaker, looking at his charges.

The uproar over the new ease and precision with which scientists can manipulate the DNA of living things has centered largely on the complicated prospect of editing human embryos. But with the federal government's approval last week of a fast-growing salmon as the first genetically altered animal Americans can eat, a menagerie of gene-edited animals is already being raised on farms and in laboratories around the world — some designed for food, some to fight disease, some, perhaps, as pets.

Just this week, researchers reported having edited mosquitoes so that they will no longer carry the parasite that causes malaria. And the power to reshape other species, scientists and bioethicists say, raises questions that are both unique to animals and may bear on the looming prospect of fiddling with our own.

"We're going to see a stream of edited animals coming through because it's so easy," said Bruce Whitelaw, a professor of animal biotechnology at the Roslin Institute at the University of Edinburgh. "It's going to change the societal question from, 'If we could do it, would we want it?' to, 'Next year we will have it; will we allow it?' "

Animal breeders have for centuries scoured species for desirable traits and combined them the old-fashioned way, by selective mating. But that process can take decades to achieve a particular goal, like cows that are both resistant to disease and produce a lot of milk. And until recently, genetic engineering techniques used to manipulate DNA had been so imprecise as to make them too expensive and difficult to perform in many animals.

But the new techniques, collectively called "gene editing" to reflect the relative ease of their use, have made all manner of previously impossible or impractical goals sufficiently fast and cheap for many to find worth pursuing. Using enzymes that can be directed to cut DNA at specific locations, they allow scientists to remove and replace bits of genetic code more or less on demand. "It's like a find-replace function in the genome of these animals," said Scott

Fahrenkrug, the chief executive of Recombinetics, based in St. Paul. “It allows us to find the natural variation that exists across a species and quickly bring it under one hood.”

At Roslin, for instance, Dr. Whitelaw has changed three genes in domesticated pigs vulnerable to African swine fever, which can devastate herds, to resemble those from wild pigs that are resistant to the disease. He is now breeding them to put them to the test.

With a tool called Talens, Recombinetics says it has created gene-edited pigs that can be fattened with less food and Brazilian beef cattle that grow large muscles, yielding more meat that may also be more tender. Others are working on chickens that produce only females for egg-laying and cattle that produce only males, since females are less efficient at converting feed to muscle.

Chinese researchers have produced meatier cashmere goats that also conveniently grow longer hair for soft sweaters, miniature pigs lacking a growth gene to be sold as novelty pets and bulky beagles lacking a muscle-inhibiting gene, an edit that could make for faster dogs.

Using the most powerful of the new tools, called Crispr-Cas9, in pursuit of treatments for human disease, researchers are also altering pigs in hopes of making them grow human organs and creating “gene drives” that would ensure that the edit to make mosquitoes malaria-proof, for instance, would spread through the whole population.

An Accelerating Pace

But the rapid advent of gene-edited animals threatens to outstrip public discussion of their risks and benefits, some scientists and bioethicists have warned.

“This essay is, in essence, a plea — let’s not ignore the nonhuman part of

the biosphere," Alta Charo of the University of Wisconsin and Henry T. Greely of Stanford University cautioned in an article titled "Crispr Critters and Crispr Cracks," to be published in The American Journal of Bioethics next month. "Not only is it much larger than the human part, but it is much more susceptible to unobserved or unfettered — but not unimportant — changes."

The discussion of gene-edited animals in farming, in particular, will most likely be colored by the existing debate over the merits of genetically engineered food, which for decades has largely centered on corn and soybeans, altered with older technology to resist pests and tolerate herbicides. Opposition to such crops, known as genetically modified organisms, or GMOs, has prompted some retailers to decline to sell food made with them, and efforts to pass legislation to label them, even as farmers have widely embraced them and scientific organizations have said they are as safe for human health and the environment as conventional crops.

Many of the new generation of edited animals do not contain DNA from another species, a frequently cited concern among opponents of genetically engineered foods, which incorporate genes from bacteria. But some consumer advocates say it may be even more difficult to reach consensus on what, if anything, should be done to the DNA of animals.

"Animals on some level will always be more controversial," said Greg Jaffe, director of biotechnology for the Center for Science in the Public Interest, a nonprofit consumer advocacy group. "If only because people think of them as closer to humans."

Advocates of the technology argue that it can make farming more efficient to help feed a growing world population with less of a toll on the environment. One projection published in a leading animal breeding journal, Genetics Selection Evolution, suggests that genome-editing could significantly increase the efficiency the livestock industry is able to achieve through conventional breeding within the same time period.

Today's chickens, for instance, produce nearly 80 percent more meat for the same amount of feed as the chickens of the 1950s; if chicken breeders had had access to genome technology over that time, said John Hickey, a quantitative geneticist and a co-author of the paper, farmers would have been able to achieve that increase and also be able to grow chickens on half the land.

Others say the technology could benefit human health. The National Science Foundation is underwriting an effort to create dairy cattle that can resist a parasite that causes sleeping sickness in sub-Saharan Africa, a blight often treated with an antimicrobial drug that ended up making its way into the meat consumed by humans.

Several projects underway to edit genetic resistance to a variety of diseases in livestock could theoretically reduce the overuse of antibiotics, which has made it harder to treat human bacterial infections. With funds from the United States Department of Agriculture, Bhanu Telugu, a University of Maryland researcher, is trying to design pigs so they can no longer serve as a reservoir for the flu virus. He argues for genome editing on behalf of animal health, too. "If we know we can eliminate the disease and we don't, it is in my mind animal cruelty," he said.

Fallout in the Food Chain

Still, some consumer advocates urge caution in applying techniques that are still so new to animals that will be consumed as food. Gene-editing tools are known to sometimes make changes to genes other than their intended targets, raising flags about how the changes might affect an animal's health or the composition of milk or meat.

"You are reducing the universe of potential risks by moving into these techniques," said Doug Gurian-Sherman, a senior scientist at the Center for Food Safety, a consumer advocacy organization that has been at the forefront of opposition to genetically engineered plants and animals. "But that is not to say we should not still proceed with great caution."

And some animal rights advocates say gene-editing is simply a means to prop up an industry that causes animals to suffer.

“Even if they can point to good intentions, it’s just exacerbating the problem,” said David Byer, a spokesman for People for the Ethical Treatment of Animals. The organization, which has urged the dairy industry to stop the practice of dehorning cattle, does not support gene-editing as a solution.

“People should stop consuming dairy or meat or eggs, not further manipulate animals by playing with their DNA,” Mr. Byer added.

The Food and Drug Administration has not said how or whether it will regulate the gene-edited animals to come. But even with the government’s stamp of approval, biotechnology advocates know that farmers are unlikely to embrace technology if they fear consumers will reject it.

And it has not helped the popularity of genetically engineered crops that their chief benefits so far — easier control of weeds and pests for corn and soybean farmers — are not terribly compelling to the eating public.

That is one reason Recombinetics has begun to show off its hornless calves.

Dehorning, which involves burning off horn-buds to stop the flow of blood to the horn tissue, has already garnered a degree of popular concern. Videos of the burning procedure carried out on Holsteins, the black-and-white breed largely responsible for the nation’s milk supply, and circulated by animal rights groups, draw long strings of critical comments.

“We know there’s a negative public perception of dehorning, and it’s certainly not a fun chore for the farmers,” said Lindsey Worden, the executive director for genetics at the Holstein Association.

A small fraction of Holsteins are naturally hornless, and several companies, including General Mills, Dannon and Walmart, have encouraged

their dairy suppliers to increase their population through conventional breeding. Farmers have made some headway, with the population of hornless Holsteins climbing to about 4 percent last year from 3 percent in 2013.

But it is slow going. That is why several dairy breeders say they are keeping tabs on Recombinetics' two hornless calves, which have just been shipped to the University of California, Davis, to be monitored for their health. There, in a few months, their sperm will be harvested, each with edited DNA, which will be used to create a new generation of hornless cattle.

Whether they will become commonplace or remain curiosities may depend largely on how the public comes to view gene editing and its various applications.

"Sometimes you can have nice benefits for animals and farmers and society but still have controversy among consumers," said Jamie Jonker, vice president for sustainability and scientific affairs at the National Milk Producers Federation. "I think dairy farmers are going to want to see how this is interpreted by the general public."

A version of this article appears in print on November 27, 2015, on page A1 of the New York edition with the headline: Open Season Is Seen in Gene Editing of Animals.

The New York Times | http://nyti.ms/1Hd41sA

SCIENCE

Jennifer Doudna, a Pioneer Who Helped Simplify Genome Editing

Profiles in Science

By ANDREW POLLACK MAY 11, 2015

BERKELEY, Calif. — As a child in Hilo, one of the less touristy parts of Hawaii, Jennifer A. Doudna felt out of place. She had blond hair and blue eyes, and she was taller than the other kids, who were mostly of Polynesian and Asian descent.

"I think to them I looked like a freak," she recently recalled. "And I felt like a freak."

Her isolation contributed to a kind of bookishness that propelled her toward science. Her upbringing "toughened her up," said her husband, Jamie Cate. "She can handle a lot of pressure."

These days, that talent is being put to the test.

Three years ago, Dr. Doudna, a biochemist at the University of California, Berkeley, helped make one of the most monumental discoveries in biology: a

relatively easy way to alter any organism's DNA, just as a computer user can edit a word in a document.

The discovery has turned Dr. Doudna (the first syllable rhymes with loud) into a celebrity of sorts, the recipient of numerous accolades and prizes. The so-called Crispr-Cas9 genome editing technique is already widely used in laboratory studies, and scientists hope it may one day help rewrite flawed genes in people, opening tremendous new possibilities for treating, even curing, diseases.

But now Dr. Doudna, 51, is battling on two fronts to control what she helped create.

While everyone welcomes Crispr-Cas9 as a strategy to treat disease, many scientists are worried that it could also be used to alter genes in human embryos, sperm or eggs in ways that can be passed from generation to generation. The prospect raises fears of a dystopian future in which scientists create an elite population of designer babies with enhanced intelligence, beauty or other traits.

Scientists in China reported last month that they had already used the technique in an attempt to change genes in human embryos, though on defective embryos and without real success.

Dr. Doudna has been organizing the scientific community to prevent this ethical line from being crossed. "The idea that you would affect evolution is a very profound thing," she said.

She is also fighting for control of what could be hugely lucrative intellectual property rights to the genome editing technique. To the surprise of many, the first sweeping patents for the technology were granted not to her, but to Feng Zhang, a scientist at the Broad Institute and M.I.T.

The University of California is challenging the decision, and the nasty

skirmish has cast a bit of a pall over the field.

“I really want to see this technology used to help people,” Dr. Doudna said. “It would be a shame if the I.P. situation would block that.”

The development of the Crispr-Cas9 technique is a story in which obscure basic biological research turned out to have huge practical implications. For Dr. Doudna, though, it is only one accomplishment in a stellar career.

“She’s been a high-impact scientist from the time she was a graduate student,” said Thomas Cech, a Nobel laureate and professor of chemistry and biochemistry at the University of Colorado, for whom Dr. Doudna was a postdoctoral researcher. “New topics, new fields of science, but she just has a knack for discovery.”

A ‘Dumbstruck’ Moment

Dr. Doudna was 7 when she moved to Hilo, where her father taught literature at the University of Hawaii campus there, and her mother lectured on history at a community college. Their daughter loved exploring the rain forests and was fascinated by how things worked. She found her calling in high school after hearing a lecture by a scientist about her research into how normal cells became cancerous.

“I was just dumbstruck,” Dr. Doudna recalled. “I wanted to be her.”

After studying biochemistry at Pomona College in California, she went to Harvard for graduate school. There her adviser, the future Nobel laureate Jack Szostak, was doing research on RNA. Some scientists believe that RNA, not DNA, was the basis of early life, since the molecule can both store genetic information and catalyze chemical reactions.

Dr. Doudna earned her doctoral degree by engineering a catalytic RNA that could self-replicate, adding evidence to that theory. But her inability to

visualize this catalytic RNA hindered her work.

So as a postdoctoral researcher in Colorado, she decided to try to determine the three-dimensional atomic structure of RNA using X-ray diffraction — and succeeded, though she had had no formal training in the technique. Structural and biochemical studies of RNA in action have been her forte ever since.

In 2000, while on the faculty at Yale, she won the Alan T. Waterman Award, given each year by the National Science Foundation to an exceptional young scientist. She moved to Berkeley in 2002.

In 2005, Dr. Doudna was approached by Jillian Banfield, an environmental researcher at Berkeley who had been sequencing the DNA of unusual microbes that lived in a highly acidic abandoned mine. In the genomes of many of these microbes were unusual repeating sequences called "clustered regularly interspaced short palindromic repeats," or Crispr.

No one was quite sure what they did, though over the next few years scientists elsewhere established that these sequences were part of a bacterial immune system. Between the repeated sequences were stretches of DNA taken from viruses that had previously infected the bacteria — genetic most-wanted posters, so to speak.

If the same virus invaded again, these stretches of DNA would permit the bacteria to recognize it and destroy it by slicing up its genetic material. Dr. Doudna was trying to figure out exactly how this happened.

"I remember thinking this is probably the most obscure thing I ever worked on," she said.

It would prove to have wide use. At a conference in early 2011, she met Emmanuelle Charpentier, a French microbiologist at Umea University in Sweden, who had already made some fundamental discoveries about the

relatively simple Crispr system in one bacterial species.

The bacterial expert and the structural biologist decided to work together.

“It was very enjoyable, because we were complementary,” said Dr. Charpentier, who recalled sitting in her office near the North Pole while Dr. Doudna regaled her with stories about Hawaii.

Along with postdoctoral researchers Martin Jinek and Krzysztof Chylinski, the two scientists eventually figured out how two pieces of RNA join up with a protein made by the bacteria called Cas9 to cut DNA at a specific spot. The researchers also found that the two RNA pieces could be combined into one and still function.

In a eureka moment, the scientists realized that this cellular defense system might be used to edit genomes, not just kill viruses.

A specific sequence of guide RNA could be made to attach to a spot virtually anywhere on the genome, and the Cas9 protein would cleave the DNA at that spot. Then pieces of the DNA could be deleted or added, just as a film editor might cut a film and splice in new frames.

The researchers demonstrated this using DNA in a test tube. While there were other genome editing techniques, they found that Crispr-Cas9 was much simpler.

The paper describing the technique, published by the journal Science in June 2012, set off a race to see if it would work in human, plant and animal cells.

Dr. Doudna, whose expertise was in working with molecules, not cells, reported such a demonstration in human cells in January 2013. But her report came four weeks after two papers were published simultaneously, one by George Church at Harvard and the other by the Broad Institute’s Dr. Zhang.

The Patent Fight

Now the University of California and the Broad Institute are arguing before the federal patent office over whether Dr. Doudna or Dr. Zhang, who last year received the Waterman Award for young scientists that Dr. Doudna had won years earlier, was the first to invent the genome editing technique. So far, the patents have gone to Dr. Zhang.

The Broad Institute claims that the paper by Dr. Doudna and Dr. Charpentier in 2012 did not demonstrate how to alter DNA in cells with nuclei, including human cells, something requiring the inventive steps that Dr. Zhang took. His patent application included pages from a lab notebook he said demonstrated that he was doing Crispr genome editing even before the 2012 paper was published.

The University of California says it filed for a patent months before Dr. Zhang did, though the Broad Institute says that initial application lacked necessary details. The university's request to the patent office says that once the 2012 paper laid out the recipe, it was obvious how to use it in cells. The university also says Dr. Zhang's notebook does not prove he could edit genomes before the 2012 paper.

Patent disputes are often settled in time. In any event, Dr. Church of Harvard said, before Crispr-Cas9 could be used to treat disease, it would need important refinements from many other researchers.

"It's going to be hard to use Feng's without Jennifer's, and it would be hard to use either of them without further improvements," he said.

The scientists have formed competing companies with rights to their patents and pending patents. Dr. Doudna co-founded Caribou Biosciences to work on research uses of Crispr-Cas9, and more recently, Intellia Therapeutics to work on disease treatments.

Dr. Church and Dr. Zhang are co-founders of Editas Medicine, which Dr. Doudna also helped start but then withdrew from. Dr. Charpentier, who is now at the Helmholtz Center for Infection Research in Germany, helped start Crispr Therapeutics. She and Dr. Doudna remain friends, but no longer collaborate on research.

Even before the dust settles, researchers are moving ahead. While contending with the patents, Dr. Doudna began hearing reports that researchers were trying to use Crispr-Cas9 to make inheritable DNA changes in embryos. Genetically altered monkeys had already been created in China using the technique.

"It's very far afield from the kind of chemistry I think about and know about," she said. Still, she felt it would be irresponsible to ignore the rumors.

She organized a meeting of leading biologists in Napa, Calif., in January. In a subsequent commentary published in Science, the group called for a moratorium on attempts to create altered babies, though they said basic research on inheritable changes should still be done.

Dr. Doudna said it was not practical to prohibit basic research. "You can't really put a lid on it, even if you wanted to," she said. She and others are trying to organize a bigger international meeting with participants from companies and governments as well as universities, possibly to set new guidelines.

Learning to Live With Fame

She is also trying to cope with her newfound quasi-celebrity status. She has been invited to hobnob with entrepreneurs in Silicon Valley, to speak to science fiction writers, to advise Hollywood on science-themed movies. The garden, her hobby, has had to wait.

In November, Dr. Doudna and Dr. Charpentier were each awarded $3 million Breakthrough Prizes, endowed by leading Internet entrepreneurs.

They accepted their awards at an Oscars-like black-tie affair attended by movie stars like Cameron Diaz and Benedict Cumberbatch. Recently Time magazine listed the two scientists among the 100 most influential people in the world.

Dr. Doudna, who has a 12-year-old son, Andrew, also finds herself a role model for women in science. Her secret: “I have a great partner,” with whom she shares the chores.

Her husband, Dr. Cate, is also a professor at Cal-Berkeley. The couple have adjacent offices, with views of the Golden Gate Bridge in the distance. Dr. Cate also studies RNA; there is some overlap, but mostly they do their own research. Andrew walks to their office from his middle school each afternoon and hangs out until his parents are ready to go home.

“I don’t think of myself as a role model, but I can see that I am,” Dr. Doudna said. “I still think of myself as that person back in Hawaii.”

A version of this article appears in print on May 12, 2015, on page D1 of the New York edition with the headline: The Gene Editor.

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STAT

Meet one of the world's most groundbreaking scientists. He's 34.

In the Lab

Meet one of the world's most groundbreaking scientists. He's 34.

Dom Smith, Matthew Orr/STAT

CRISPR is a powerful gene-editing tool with transformative potential. Feng Zhang, a scientist at the Broad Institute, explains how it works.

By Sharon Begley @sxbegle

November 6, 2015

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As the dish of steamed chicken feet clattered onto the table, an impish toddler drummed with her chop sticks. Nobody in the noisy restaurant in Boston's Chinatown gave a second glance at the man dressed in a polo shirt and jeans enjoying dim sum with his little girl, wife, and mother.

No one could have guessed that Feng Zhang, at 34, is widely considered the most transformative biologist of his generation, a double threat to win a Nobel Prize in the near future. Or that his discoveries could finally bring cures for some of the greatest causes of human suffering, from autism and schizophrenia to cancer and blindness. Or that he has touched off a global furor over the possibility that a genetics tool he developed could usher in a dystopian age of designer babies.

At that moment, Zhang was simply a young father, husband, and son struggling to explain what drives him, why it isn't unusual for him to arrive home from his lab at 1 or 2 or even 3 in the morning.

He thinks it's important, he told a reporter he had invited to join him for brunch. He enjoys it, he wants to carry on the work of mentors who invested in him, he … "The autumn leaves," his mother, Shujun Zhou, piped up.

Read more: Global summit opens door to controversial gene-editing of human embryos

Zhang was 11 when he and his mother left China and settled in Des Moines, Iowa. A few years later, when he was in high school, she often waited in her car for hours while he worked late in a gene therapy lab. Driving home in the gathering dark one autumn evening, mother and son were struck by the sight of falling leaves, dead and dying after lives measured in mere months. They spoke about how little time anyone has, she recalled, and how easy it is for a life to disappear without the slightest trace that it had ever been. "It just seemed important to me to try my best to make a difference," Zhang said.

As much as anyone in science, he already has.

STAT has followed Zhang since the summer, accompanying him to standing-room-only talks, interviewing his mentors and lab trainees, and spending hours with Zhang as he talked about his life in more detail than he previously has in public. What emerged is a portrait of a mild-mannered scientist with a brash vision, a striver with an immigrant's ambition to scale the greatest heights in his adopted land, and a researcher who is impatient with the plodding ways of his craft.

Colleagues note his ability to identify promising ideas early, to stoke the creative fires of his junior lab members, and to resist the temptation to pursue likely-to-succeed but incremental advances and instead to take risks. When a member of his lab proposes a project, Zhang asks: Will it be a "hack," clever but inconsequential, or an innovation?

Zhang helped create two revolutionary genetic and neuroscience technologies. As a graduate student, he was a key member of the team that figured out how to light up neurons in the brain, allowing scientists to unravel which circuits control which behaviors and search for the roots of mental illnesses such as schizophrenia and bipolar disorder. Just a

few years later, Zhang made the discovery that would vault him into the front ranks of the world's biologists: how to edit the genomes of plants and animals—including humans—quickly, easily, and efficiently.

The tool is already being used in the lab to make human cells impervious to HIV; cure mice of muscular dystrophy, cataracts, and a hereditary liver disease; and improve crops including rice, tomatoes, oranges, tobacco, and wheat. But it also could be used to modify genes in human eggs, sperm, and embryos, raising the specter of parents choosing their baby's traits — personality, athletic ability, looks — like options on a Lexus.

Called CRISPR-Cas9, the technology quickly spawned three companies with hundreds of millions of dollars in venture financing and opened a new era in molecular biology.

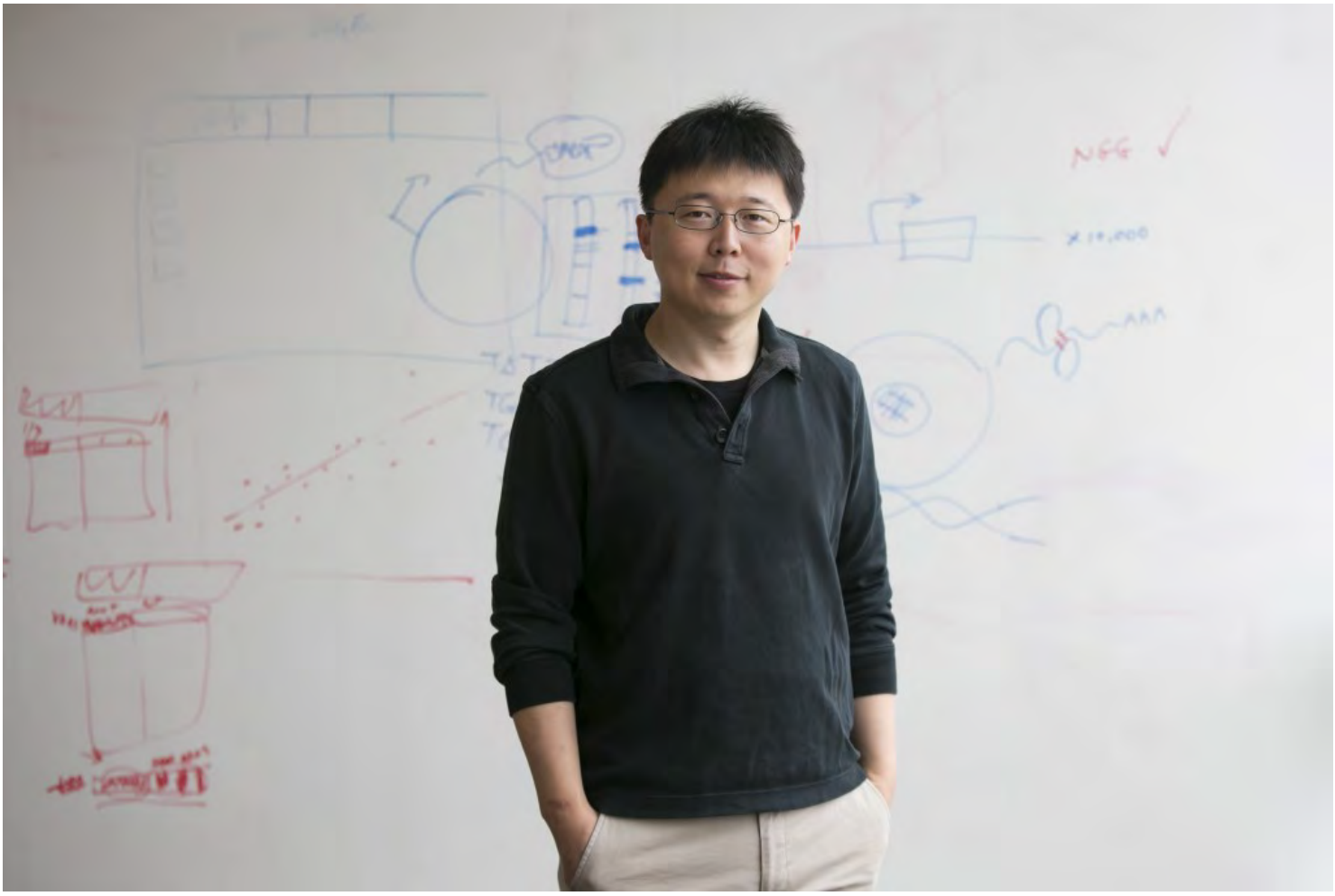
It's "changing how we do science," said MIT biologist Phillip Sharp, who shared the 1993 Nobel prize in medicine.

The gene editing tool is so powerful — with such immense implications for the environment and humanity — that science organizations from around the world are convening a global forum next month to craft guidelines for using it responsibly.

Zhang is the youngest head of a lab at the Broad Institute in Cambridge, Mass., a high-powered genomics research center affiliated with MIT and Harvard, where he is one of only eight "core faculty." Many of his post-doctoral fellows and graduate students are older. Rarely without a smile, he regularly bounds into the office of the Broad's director, Eric Lander, showing him his latest "cool" data.

Exactly how much credit Zhang deserves for the development of CRISPR is the focus of a bitter patent fight, but if he and the Broad prevail, he stands to become the latest of MIT's wealthy scientist-entrepreneurs. It's a future Zhang could never have imagined as a child in Iowa. After arriving from China, he and his mother initially got by on what she earned in menial jobs such as a motel housekeeper — though she was a computer engineer. His father, an administrator at a science and technology university, did not join them for several years.

Then his life changed thanks to the most mundane of experiences. Zhang went to the movies.



Katherine Taylor/STAT

Feng Zhang at the Broad Institute in Cambridge, Mass.

Life might be programmable

In Des Moines, middle-school biology class meant dissecting frogs stinking of formaldehyde. But Zhang was rescued by a Saturday enrichment program in molecular biology, where the instructors, not being fools, figured that a reasonable way to keep a bunch of teenagers engaged was to show them *Jurassic Park*.

"Both of my parents work in computer science, so I was always interested in programming," Zhang recalled. The 1993 film, in which hubristic researchers merged dinosaur and frog DNA to bring back the extinct reptiles, "told me that biology might also be a programmable system."

A seed had been planted in his mind. An organism's genetic instructions, he realized, could be overwritten to change its characteristics, just as his parents wrote computer code.

He got his first chance to program a living thing in 1995, as a sophomore at Theodore Roosevelt High School. The head of a program for gifted students asked Zhang if he'd like to volunteer after school in a gene therapy lab at nearby Methodist Hospital. "I said it sounds great," Zhang recalled, and although he "knew zero" about advanced biology, the head of the lab, Dr. John Levy, didn't blanch at his inexperience.

Every afternoon, Levy would sit in a break room drinking tea and scribbling on a pad to explain concepts in molecular biology. Zhang was a quick study, learning key techniques and succeeding in his warm-up project: using viruses to slip jellyfish genes for a glow-in-the-dark molecule called green fluorescent protein into human melanoma cells.

It wasn't resurrecting dinosaurs, but he had engineered cells of one species to express genes from another, and the eerie emerald light emanating from the cells was proof. "They glowed!" Zhang recalled with an excitement he still retains 20 years later.

Zhang spent the rest of the year studying whether the fluorescent protein, which absorbs ultraviolet light, might protect DNA from the damaging and cancer-causing effects of ultraviolet light. He discovered that it does, and the experiment became his Iowa state science fair project, which had the added attraction of drawing "kids like me," Zhang said: "geeky."

He did another genetics project under Levy's mentorship his junior year, in viruses, which earned him third place nationally and a $50,000 scholarship in the 2000 Intel Science Talent Search competition.

It "made me want to go out and cure HIV," Zhang said. That wasn't exactly in the cards for a high school student; neither was extending his fluorescent protein work to see if, by blocking ultraviolet light, it could help prevent melanoma. But he had learned a valuable lesson: intriguing scientific discoveries often go nowhere.

Admitted to Harvard with a full scholarship, Zhang conducted influenza-virus research in the lab of chemist Xiaowei Zhuang while majoring in chemistry and physics. The research led to a 2004 paper in a top journal on how flu viruses enter cells. Key to the discovery: the glowing jellyfish protein Zhang had first played with in Iowa.

Zhang was a bit of a Julia Child in the lab, able to get wondrous results but prone to the laboratory version of dropping turkeys onto the floor. In organic chemistry, he forgot that putting acid into a certain hot reaction is a no-no. "Everything foamed up and exploded inside the chemical hood," he recalled. He and his partner fled.

Another experience had a more lasting impact. When a close friend and fellow student developed major depressive disorder, Zhang spent hours trying to help and making sure he was not suicidal. The friend was so deep in the abyss of depression as to be unreachable, however, and had to take a year off from Harvard. Zhang was deeply touched and dedicated himself to developing better treatments for mental illness.

Zhang was a bit of a Julia Child in the lab, able to get wondrous results but prone to the laboratory version of dropping turkeys onto the floor.

Albert Einstein is renowned for having published five mind-bending discoveries in a single year. Zhang was about to embark on a period almost as fertile. After graduating from Harvard in June 2004, Zhang headed to graduate school at Stanford, joining the lab of a rising young neuroscience professor named Karl Deisseroth. With graduate student Ed Boyden, the trio invented optogenetics, in which light-sensitive proteins are slipped into neurons so light can activate specific neural circuits. Zhang's contribution was developing a system for using viruses to ferry foreign genes into neurons and get the genes to churn out light-sensitive proteins.

In 2007, with reporters scheduled to visit, Deisseroth had Zhang engineer a mouse with light-sensitive neurons in its motor cortex. Sure enough, light turned on the neurons and the mice walked in circles. Today, optogenetics is considered one of the seminal achievements in neuroscience, used by researchers worldwide to map neural circuitry, including that underlying schizophrenia, depression, or autism.

Doctorate in hand, Zhang "started thinking about how I could insert genes easily into animals," much as he did for optogenetics but in ways that would work for every kind of animal and every kind of gene. In 2009 he received a position at Harvard's Society of Fellows, a prestigious perch "for people who are preternaturally independent and creative," said Broad neuroscientist and former Harvard provost Steven Hyman. "Feng is both."

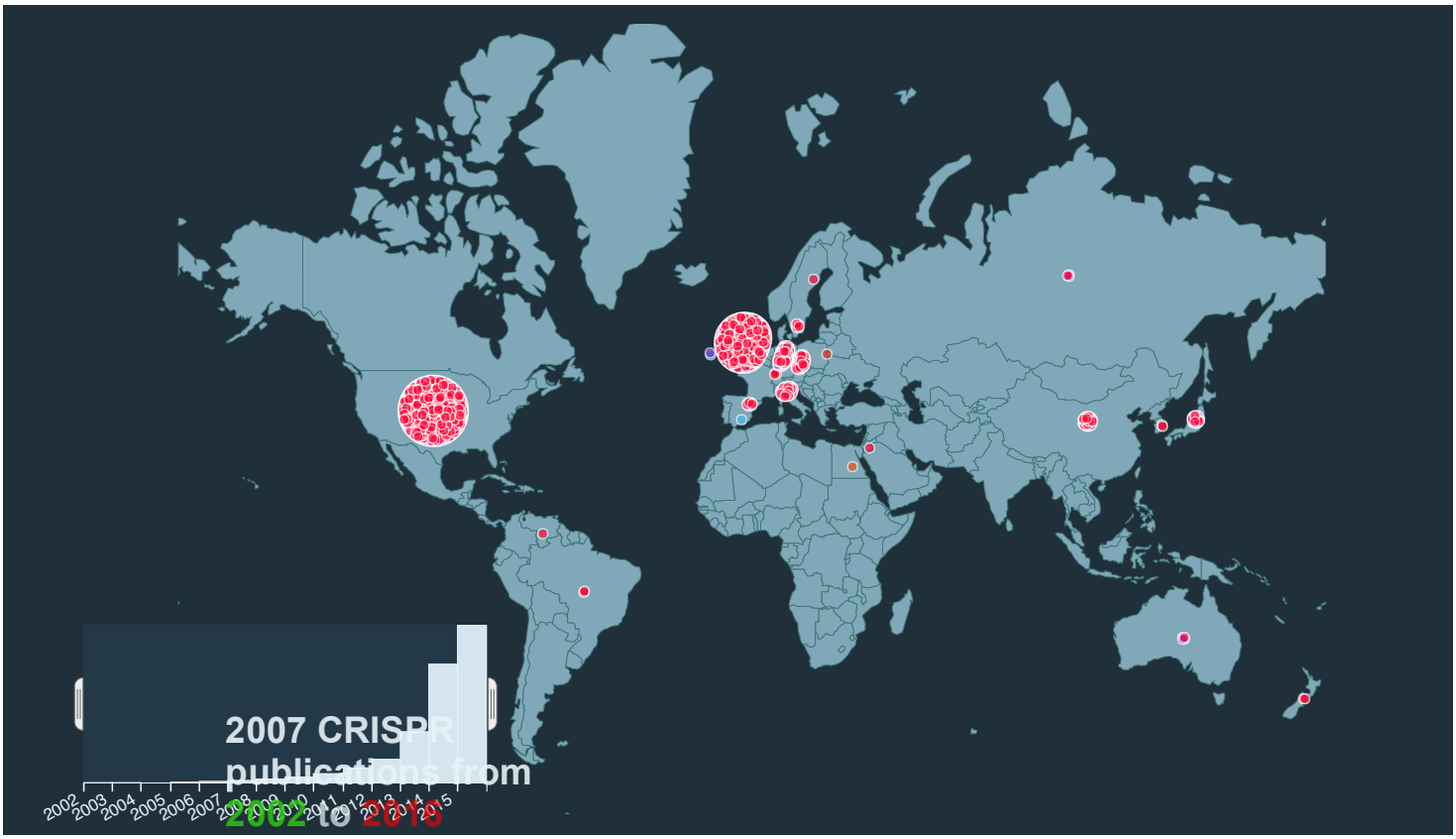
Read more: Gene drive gives scientists power to hijack evolution

The position didn't come with a lab, however. Zhang begged and borrowed space in labs of senior Harvard scientists. He began with the then-leading gene editing technique, in which proteins studded with structures called "zinc fingers" recognize a specific DNA sequence and cut it. Cells naturally repair such cuts and, if foreign DNA has also been slipped into the cell, incorporate the substitute DNA. Presto: an edited genome. The trouble is, zinc fingers are "remarkably difficult to work with," Zhang said.

Scientists unveiled another gene-editing technique, called TALEs, in 2009. But TALEs, like zinc fingers, are difficult-to-make proteins. "I was teaching students to build TALEs and it would take three months before they could even use it," Zhang recalled. He was the lead author on a study that involved creating TALEs able to home to specific DNA sequences in human and mouse cells and turn genes on or off. But he wasn't satisfied. "I thought there must be better ways to do gene editing," he said.

His Fellows appointment nearing an end, Zhang needed a job. A neuroscientist at MIT's McGovern Institute for Brain Research had heard Deisseroth sing the praises of "this amazing superstar," recalled McGovern director Robert Desimone. In a collaborative enterprise like science, where it's not unusual for papers to have a dozen authors, "you always wonder who did what," Desimone said. The McGovern asked around and was assured "that Feng played a key role" in developing optogenetics, said Desimone. Zhang's list of papers, he added, "was the strongest publication history of anyone [at this stage of a career] in the history of neuroscience." Zhang was hired, by both MIT and the Broad.

In February 2011, a visiting scientist told a meeting of the Broad's advisory board about his research on bacterial genomes containing an immune system called CRISPR. "I was sitting in the back of the room and my mind had been drifting," Zhang recalled, but the odd acronym immediately sparked his curiosity.



(Visualization by Natalia Bronshtein/STAT; Source: PubMed)

Drag timeline handles to filter CRISPR studies by year, and hover over or click the colored circles to see publication details.

“I had no idea what CRISPR was, but I looked it up on Google and became really excited. Fortunately, the field was young and there were not a lot of papers to read.” He spent much of a scientific meeting in Miami a few days later holed up in his hotel room poring over CRISPR papers.

What he learned was that CRISPR — Clustered Regularly Interspaced Short Palindromic Repeats — had been discovered by microbiologists in bacteria, where they defend against viruses. The CRISPR system consists of a search-and-destroy duo: Genetic material called RNA homes in on a specific stretch of DNA; an enzyme called Cas9 cuts that DNA. Since CRISPR can destroy viruses “that make yogurt taste funny,” Zhang said, “the field was focused on using CRISPR to make better yogurt.”

Zhang had bigger goals. “We wanted to see, ‘Can we make it work in human cells?’,” he recalled. He e-mailed graduate student Le Cong: “This could be really big.”

It was an audacious aim. Sticking to TALEs—a more established technology—was the safer course, Cong reflected later, but “we decided to give CRISPR a try. It was worth taking the risk.”

Katherine Taylor/STAT

Feng Zhang arrives for work at the Broad Institute.

Working like crazy

Back in Cambridge, Cong "immediately recognized why Feng was excited," he said. TALEs had driven them crazy, as they laboriously synthesized protein after protein only to find that it wouldn't home in on the stretch of DNA they wanted. But CRISPR used RNA, not proteins, to recognize specific DNA sequences in a genome. If synthesizing proteins is as complicated as making a roller coaster out of K'nex, then constructing RNAs is as simple as stringing beads on thread.

Instead of warming up by studying CRISPR in bacteria, as other scientists were, the duo jumped to human and mouse cells, figuring that only if CRISPR worked in these higher-order cells would it be medically important. On a whiteboard in his office, Zhang listed individual experiments they would need to do and split them up.

"It was initially Feng and myself, and we were working like crazy," Cong said. The scientists spent months testing Cas9 enzymes, in particular monitoring whether they got to the nuclei of human cells, where the genes reside. Bacteria, where the CRISPR system originated, do not have nuclei, so there was no guarantee that it would work in cells that do. "We wanted to show that CRISPR was better than TALEs, that it was revolutionary and the system of choice for genome editing," Cong said.

They often worked until 11 p.m. or later — Zhang had classes to teach, and couldn't start his experiments until late afternoon. They took breaks for ramen noodles, Chinese take-out or burritos and, once, to crash a party at Zhang's apartment complex and try their first tequila shots. (Only one each; they returned to the lab that night, too.)

The scientists wanted to demonstrate at least two crucial things: that CRISPR edited the genome in mouse and human cells, and that the edited genome functioned. They'd targeted the gene for green fluorescent protein, Zhang's sentimental favorite from high school, and used microscopes and fancy cameras to study the green glow: The less the cells glowed green, the more CRISPR had edited out the fluorescence gene.

Once the basics worked, by the spring of 2012, they had enough data for a paper, Zhang said. But it would have been only a so-so paper. "I didn't want to submit the paper just because the result was publishable," he said. "I want to wait until we have a paper that can make a significant difference, not just to be first with something."

"We thought we had the luxury of time," Cong recalled. "We didn't know about the competition."

But competition there was. In June 2012, scientists led by Emmanuelle Charpentier, then at Umea University in Sweden, and Jennifer Doudna of the University of California, Berkeley, reported using CRISPR-Cas9 to cut target DNA sequences in test tubes, raising the "potential . . . for RNA-programmable genome editing," they wrote in their paper in the journal Science.

Zhang didn't feel he had been scooped, he said: many biochemical tricks work in test tubes but fail in human cells. Before the Charpentier-Doudna paper was published, Cong recalled, they had come up with "a completely independent and different way of using Cas9 for genome editing than the strategy proposed" in that June paper: "We had these details figured out before [it] was published," Cong said, and Zhang included those details in grant applications he submitted, also before June.

Moreover, when they read their rivals' paper, Cong said, they saw that it described the use of two molecules that were "very different" from the CRISPR-Cas9 system Zhang's team had designed and lacked "critical components" for making the genome-editing system work in living cells as opposed to test tubes.

The team therefore pressed on through late summer, amassing data showing their system could not only target genes inside human and mouse cells but edit several at once. During the final sprint, Zhang recruited additional members of his growing lab, an approach his colleagues compare to that at tech start-ups: he recognizes a killer app and throws bodies into the fray like a general calling up infantry. "We"—a word Zhang emphasized—"showed we could edit the human genome."

He sent the paper to Science on Oct. 5. It was published online in early January 2013, along with a similar paper from the lab of Harvard's George Church, where Zhang had

worked during his Harvard fellowship. Asked if he knew his old mentor was also in the CRISPR race, Zhang said he had no idea.

CRISPR “is changing how we do science.”

Phillip Sharp, MIT biologist and Nobel laureate

Zhang has received some bad press and is the occasional target of Twitter barbs, because MIT paid a $70 fee for accelerated review when it applied for a CRISPR patent. That has been portrayed by rivals as somehow jumping the line, since the Doudna-Charpentier patent application was submitted months earlier, but it’s not clear it made any difference in the patent decision.

At the time, the patent office used a system that awards patents to whoever first invented or conceived of something novel; Zhang has submitted lab notebooks meant to show that his lab was indeed first, which will ultimately carry more weight than the expedited review. Under the current “first to file” system, the patent might have gone to Doudna and Charpentier. But with the old first-to-invent system in effect, MIT received a key patent for the use of CRISPR to edit plant and animal genomes, with Zhang listed as inventor, in April 2014.

Berkeley has appealed that decision. The university contends that Doudna and Charpentier achieved the key CRISPR breakthroughs — in particular, identifying the three molecules that are crucial to making CRISPR work — and that Zhang’s success in animal cells was just an extension of their work.

Zhang rejects that characterization, arguing that Doudna and Charpentier’s 2012 paper “showed that you can cut DNA in test tubes.” If extending that to plant and animal cells was “obvious,” as critics of the Broad contend, “then why would our paper be published in Science?,” one of the world’s top journals, he asked. He had the idea of using Cas9 to edit animal genomes in 2011, he said, and to get it to work in human cells he used a different design of RNA than the one Doudna and Charpentier described.

Zhang’s breakthrough helped open the floodgates: The number of scientific papers with CRISPR in their title rose from 90 in 2012 to 741 (and counting) this year. That was partly Zhang’s doing: he has been using a non-profit called AddGene to distribute genetic and other material, called reagents, to biologists around the world.

The intense interest reflects the astonishing power of CRISPR for both basic and commercial research. It is the rare media mention of CRISPR that does not include the phrase “designer baby,” however. The technology works in essentially any cell, including human eggs, sperm, and embryos. Someone who developed from such “germline engineering” would carry Genome 2.0. So would his or her descendants. That has led to fevered speculation about editing genes to enhance personality, cognitive, behavioral, and physical traits.

Read more: A debate: Should we edit the human germline?

In April, when scientists in China reported using CRISPR to edit the genomes of non-viable embryos created through in vitro fertilization, it created a furor. The U.S. National Academies will hold an international summit on genome editing—its promise, risk, and need for oversight—next month.

Zhang described his work to the Academies in October, emphasizing that his lab and the company he co-founded, Editas Medicine, are developing CRISPR-based therapies for non-germline cells—editing genes in blood cells, for instance, to cure sickle-cell disease. The possibility of changing one individual at a time, he says, would be sufficiently revolutionary.

Katherine Taylor/STAT
Feng Zhang runs an experiment with DNA and RNA in his lab.

‘Everything goes faster in this lab’

More than anything, what stands out about Zhang is his productivity. Since his breakthrough CRISPR paper in 2013, he has had 38 more publications. His lab hums into the night, with Zhang often right there beside his junior colleagues, happily pipetting. “He comes back after eating dinner with his family” — his wife, his toddler daughter, and his parents squeeze into an apartment about a mile from the Broad — “because he genuinely can’t wait until morning to know the answer” from the experiment his team is running, said post-doctoral fellow Naomi Habib. “He leads by example. He doesn’t measure people’s hours, but infects us with his passion.”

When Habib told him she was expecting her second child—news that many lab heads, male and female, would greet with annoyance or even anger—Zhang arranged for a technician to get up to speed on her experiments and keep them going during her absence.

He gives credit to other scientists, even those on the bottom rungs of the lab hierarchy. When he and colleagues engineered a new CRISPR-related protein in 2014, he named it SAM—ostensibly for “synergistic activation mediators,” but really for the initials of three students who did the work. “We had to come up with a fancy name to please the reviewers,” Zhang said, “but SAM was really for them.”

He has an uncanny ability to recognize the potential of an idea, much as he did when he first heard of CRISPR. In May, a scientist attending a genome engineering meeting at the Broad mentioned that some bacteria might use DNA-cutting enzymes other than Cas9. Afterward, Zhang casually walked over to one of his graduate students, Bernd Zetsche, and asked, “Are you busy?” Zetsche um’ed and ah’ed, since of course he was in the middle of a project, but Zhang redirected him to his latest brainstorm.

By September they had a published paper describing some members of a new family of molecular scissors that can be used to edit human and other genomes. “Somehow,” said Zetsche, still somewhat at a loss to explain the astonishingly fast turnaround, “everything goes faster in this lab.”

Although Zhang is known for CRISPR, he views that as only a means to his true goal: using genetics to understand and, ultimately, treat diseases of the mind. Half his lab is focused on brain research. It’s the possibility of making a real difference in autism, depression, schizophrenia, and other serious disorders that drives him, Zhang said. All the things such illnesses take away, he said —the ability to feel joy, to make meaningful social connections, to think clearly and deeply—are “a very essential part of being human.”

At a recent lab meeting, Habib was showing three dozen people at a long conference table PowerPoint slides of results from an experiment measuring which of thousands of genes are active in which brain cells. Zhang, although not dominating the conversation, was laser-focused on making sure the significance of their findings were communicated to the public.

“Figure 1 is not punchy enough,” he said. “It would be really nice if Figure 1 said, ‘We can do this and it’s important.’ ” Think of your audience “as a high school biology class and not your peers,” he suggested.

If the world doesn’t know you made a breakthrough, he told his colleagues, then for practical purposes you didn’t.

CORRECTION: An earlier version of this story incorrectly described Zhang’s relationship with AddGene, the non-profit he is using to share CRISPR tools, and the fee the Broad Institute paid for expedited review of its patent application.

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