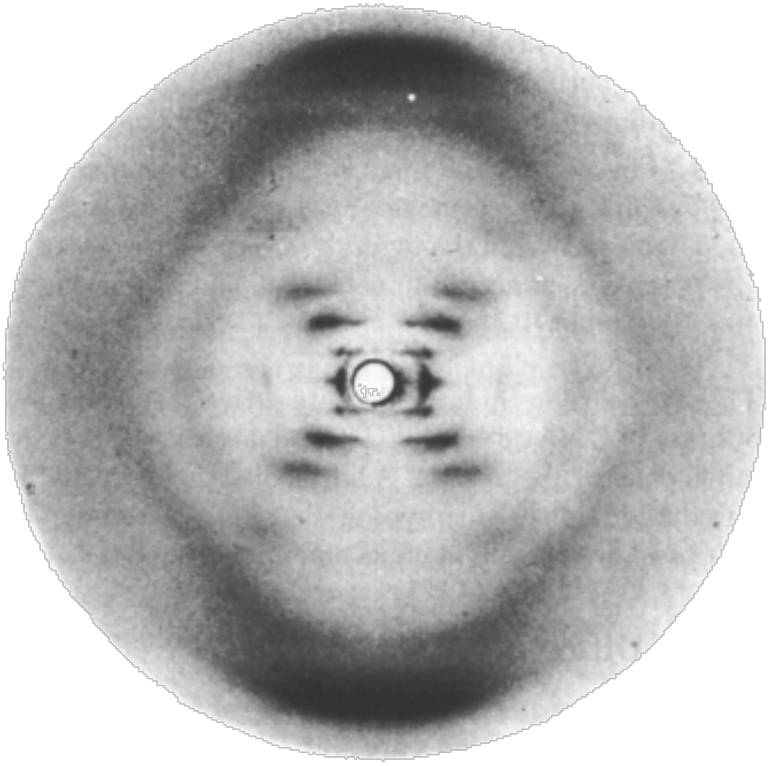
COMPASSIONATE TECHNOLOGIES

GENETIC ENGINEERING



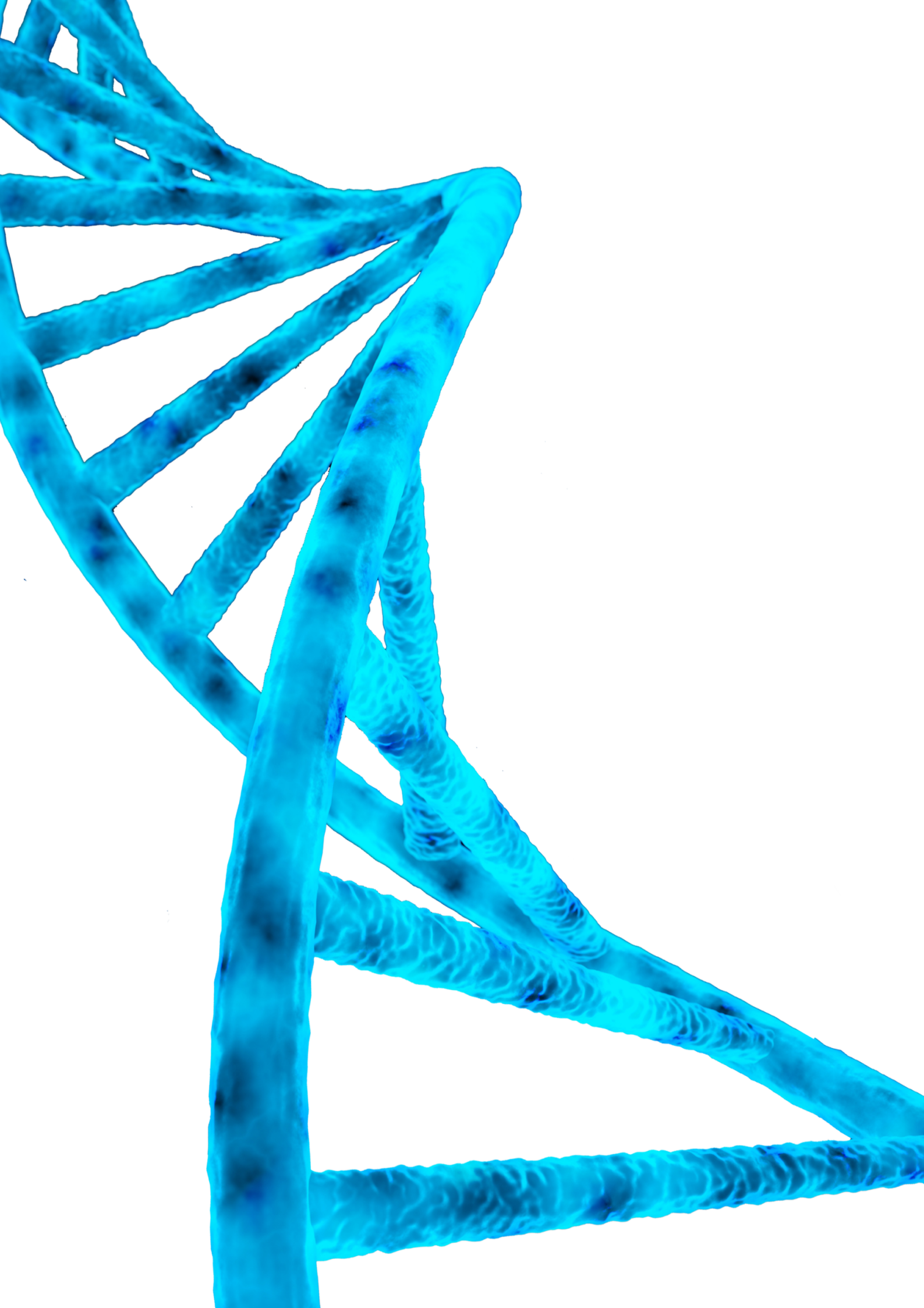
trends // research // technology // business

Curiosity becomes Science.

Tinkering becomes Technology.

Play becomes Business.

Don't wander far from your inner child. With their curiosity they will break the rules and innovate.



Decoding Racism: Fear, Bias, and Biology

Racism has long been a painful part of the human experience, from slavery and genocides throughout history, to the institutionalized caste system in India. Today, many desire to live in a post-racial society, but we face heavy histories, shame and confusion about our own natures.

Before we begin decoding racism, let's start with a working definition. In the individual human, it's a snap judgment based on race leading to violence. In a society, it can become institutionalized into segregation and in the extreme, race-based slavery; not only just denial of resources, but denial of freedom and life.

It is the epitome of non-compassion, a deadness to the suffering of others. How do we go from innocent children to taking pleasure in violently lashing and killing another human being?

On Duality: Scarcity and Collaboration

Taking many steps back from the present, life was once a collection of barely multi-cellular organisms swarming around seeking energy to complete metabolic functions. These organisms are the precursors of our own DNA. Without energy, these organisms would die.

This scarcity has been the backbone of our evolution into a human species. We compete for survival, against other animals, working both with our fellow man and against him. We love, and we murder. We share, and we steal. We welcome, and we shun.

As humans, we exist on this earth in duality. A mixing of yin and yang, dark and light, of hot and cold. Denying the existence of either extreme is a denial of ourselves, a seesaw without the other end. We can't exist.

On Fear: Racism Begins Underneath Awareness

Why would a police officer mistakenly shoot an unarmed black man? If you ask the officer he might not even know. In seeking answers, science comes to our aide, showing that racism is deeply rooted in our minds beyond our own awareness.

In a 2004 study, 13 white students who were proven to be consciously unprejudiced were subliminally shown black faces, quickly flashed for under 0.3 seconds, underneath conscious awareness. The part of their brains that control fear, the amygdala was activated. However, when shown the image for 0.525 seconds, barely long enough to be aware, the fear response was inhibited.

In the case of police actions, where life and death decisions are made in under a second, the slightest presence of racism may be enough to tip the scales so that 5x more unarmed black men are killed than unarmed white men. The study shows, though, that just because we experience race-based fear, doesn't mean that we must act on it. It begs the question, where does this fear come from?

On Confirmation Bias: Racism Has Inertia

In the 1880s the popular science of eugenics swept across the nation. Width and circumference of the skull were measured, looking for an easily measurable basis for violence, which researchers was associated with negroid features, spreading the conception that blacks were violent and less able to control their emotions. These studies led to the forced sterilization of Native American and African Americans who possessed societally undesirable features of uncontrollable violence.

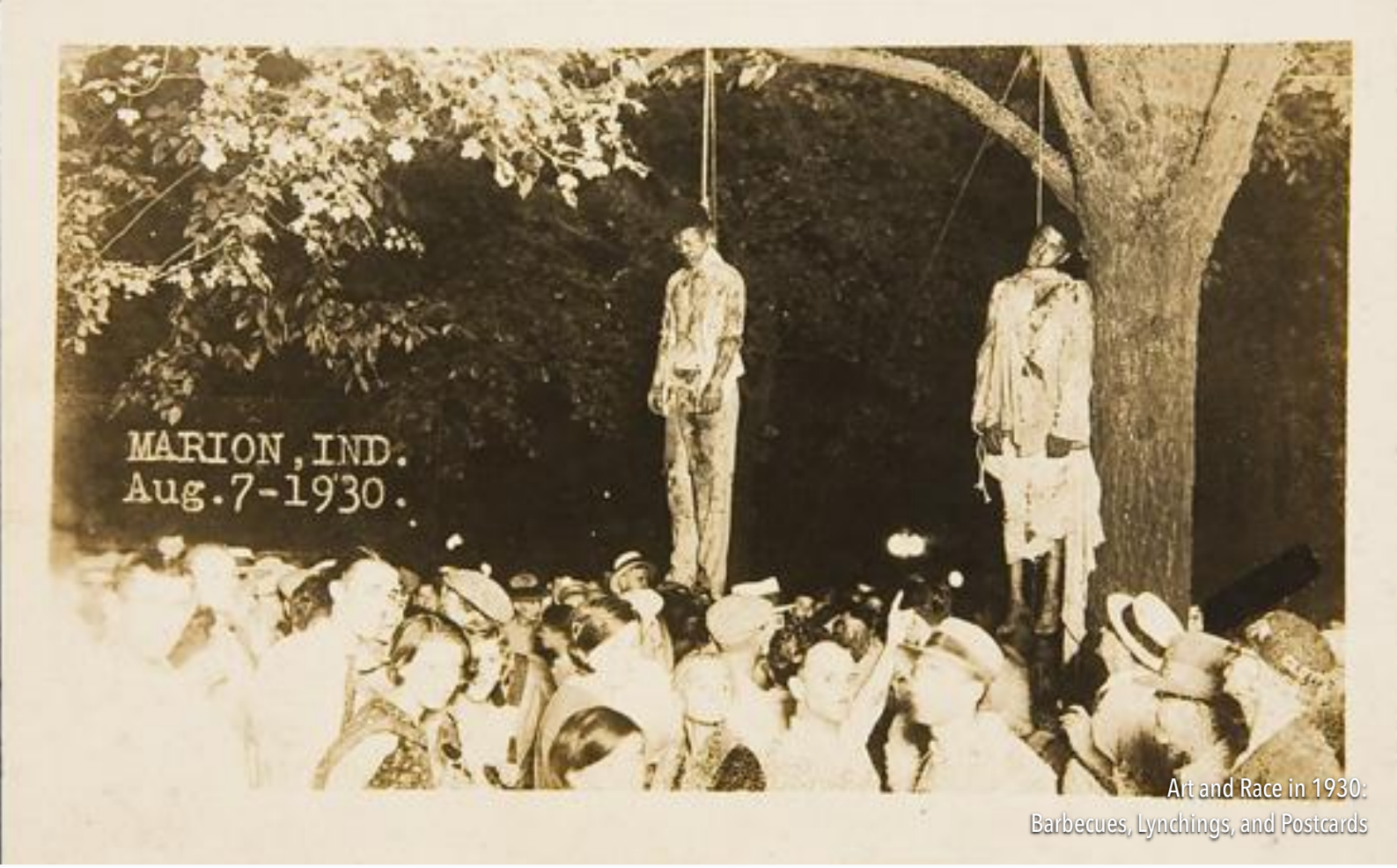
Memories become heavy over time with inertia, grooved into our minds like a deep river. **Confirmation bias** means that once our minds are set they are hard to change.

We form thoughts and biases in two ways: the first from direct experience, the second from storytelling and culture. This means that a fear can spread from generation to generation, with no direct experience, only through stories, advertising, and art - the things that make up culture.

Cultural Genetics and DNA

Through culture, stories propagate themselves like genes do. They mutate and spread from generation to generation, and they feed off of emotion, a social energy, the same way that DNA feeds off of energy for survival and replication.

Where our genes end and our culture begins is an age-old question, one that has not yet been answered by science. As we enter an era of genetic engineering, it's important to consider what effects this will have.



Art and Race in 1930:
Barbecues, Lynchings, and Postcards

Art and Race in 2017
Portraits by Angelica Daas Humanae

Building the Staircase of Life: Tinkering 10,000 Years Ago

We've been genetically engineering for over 10,000 years, taking advantage of random genetic mutations and exerting human selection in agriculture. Only in the last 50 years have we developed the tools to exert greater control and speed onto the process.

With a technique discovered in the 1970s and reliably patented just this year, we can now cut and past DNA, albeit still with very high error rates. You definitely would not want to try designing your baby - at least not yet.

Outside of science fiction and in real time, the market is now making rapid advancements in gene therapies for cancer and diseases like cystic fibrosis.

The Original Genetic Engineers: Ancient Farmers

Before we knew that genes were made of deoxyribose nucleic acid (DNA), ancient farmers already knew the concept of genes. In Mexico, for example, they knew that if they planted the seeds of the most fruitful teosinte grain, a type of grass with barely more seed than the grass that grows in your lawn, the grains would over several growing cycles get larger - and larger - until the Aztec society was dependent on maize (corn).

Not to mention that your puppy Fido has also been genetically engineered by human selection. Originally descended from the Asian wolf, aggressive pups were culled (killed) and docile pups were bred - leading to our 300 breeds of our modern day dog, ranging from Great Danes to chihuahuas.

So What Are Genes, Anyway?

The code that makes us up are made of 4 molecules represented by the letters: G, A, T, and C. These molecules form a helical structure.

Imagine a spiral staircase 750,000 miles long, tied in knots around itself, wrapping around the Earth 5 times. Every time a new cell is created, a new staircase must be built.

In order to create the proteins that make life, a man has to go step-by-step on the staircase - each step being one letter of the very long recipe that creates a specific life. Some parts of the staircase are so treacherous and knotted that the man jumps off and skips to the next part, leaving many portions of the recipe unread.

Each human has 5 billion +/- 5 million steps in their staircase (called **nucleotides** in DNA language). That's a huge range between individual humans, meaning that your neighbor might have 10 million more or fewer nucleotides than you do!

What does this code even do anyway? Does having more of less of it make you less of a human? It turns out that only 5% of our DNA actually codes for proteins, the essential ingredients of life.

The purpose of the rest is a mystery to science. Although this "junk" code is what makes familial DNA tests possible. The endless repetitive patterns in the junk code are very accurate at telling us where we came from, even from many generations ago.

Genetic Engineering Today: Gene Therapies

Over our lives, our DNA changes and mutates in the process of aging. Our bodies are very protective of our genes, and our immune system exists to detect and destroy any items that don't belong, like viruses or bacteria.

Some people's genes cause disease though, like the BRCA1/2 genes that are highly linked to breast cancer. In many cases of cancer, there is a gene that either causes greater incidence of genetic mutations or allows mutated genes to replicate wildly and out of control into tumors.

In order to bypass the body's natural defense and DNA-repair system, scientists use vectors, Trojan horses that sneak in therapeutic DNA past the defensive walls.

Viruses are common vectors (you'll hear the term **viral vector** a lot). Some therapies today use a modified version of the AIDS virus to inject genetic information, it's like a cellular needle.

Another method is by extracting our own stem cells, the "blank slate" cells which can become almost anything. The problem with this therapy is that stem cells are hard to find in an adult human, found usually in the bone marrow or roots of your teeth - two places where we really don't want doctors to go!

12,000 years ago in Asia dogs were engineered using culling and selective breeding methods, turning wolves into man's best friend

10,000 years ago in South America teosinte grain was engineered through seed selection into maize and then corn.

Scared? It might be in your genes.

How are you, a collection of nearly 30 trillion cells, all with identical DNA codes, a functioning human being? Why doesn't a skin cell divide and turn into a blood cell? The expression of your genetic code is regulated by epigenetics.

Epigenetics alters a cell's identity through interacting with the environment. Recent evidence suggests our environment may not only affect us, but our offspring as well. This way, Lamarckian evolution does not just bridge the divide between nature and nurture, but unites us and our ancestors as well.

Lamarckian Evolution vs. Darwinian Evolution

While Darwin has stolen the spotlight, Lamarck held a somewhat different theory of evolution. Jean-Babtiste Lamarck was a French soldier and naturalist who laid the foundations of modern zoology.

Lamarck thought there existed an inheritance of **acquired characteristics**, for example that the giraffe arose from horses as they stretched their necks to reach tall branches; whereas Darwin theorized that genetic changes only occurred through random genetic mutations. Though Lamarckism has been replaced with genetic inheritance and Darwinism, the idea that traits acquired during an organism's lifetime may be passed down is still possible through the discovery of epigenetics.

The Science of Epigenetics

Epigenetics describes changes in gene expression by altering how your DNA is packaged, not what it is composed of.

Each one of your thirty trillion cells must each store approximately seven feet of DNA into a cellular nucleus 600 times smaller than a fine grain of sand. To achieve this, DNA is spooled around proteins called histones that organize DNA into progressively more complex superstructures from nucleosomes through to chromatin and the familiar forty six chromosomes most of us possess. Altering how the DNA thread adheres to the histone spools will change chromosome structure and in turn, function.

DNA packaging is modified through tightening or loosening the chromatin. Loosening DNA coiling allow protein synthesis machinery to access the genes, producing proteins, tightening DNA restricts this access. This process is governed by the addition or subtraction of specific molecules onto either the histone or the DNA. Generally, adding a molecule loosens a region of the coil. These molecules are added through an interaction with the cells environment and passed down through cell lines, enabling Lamarckian evolution.

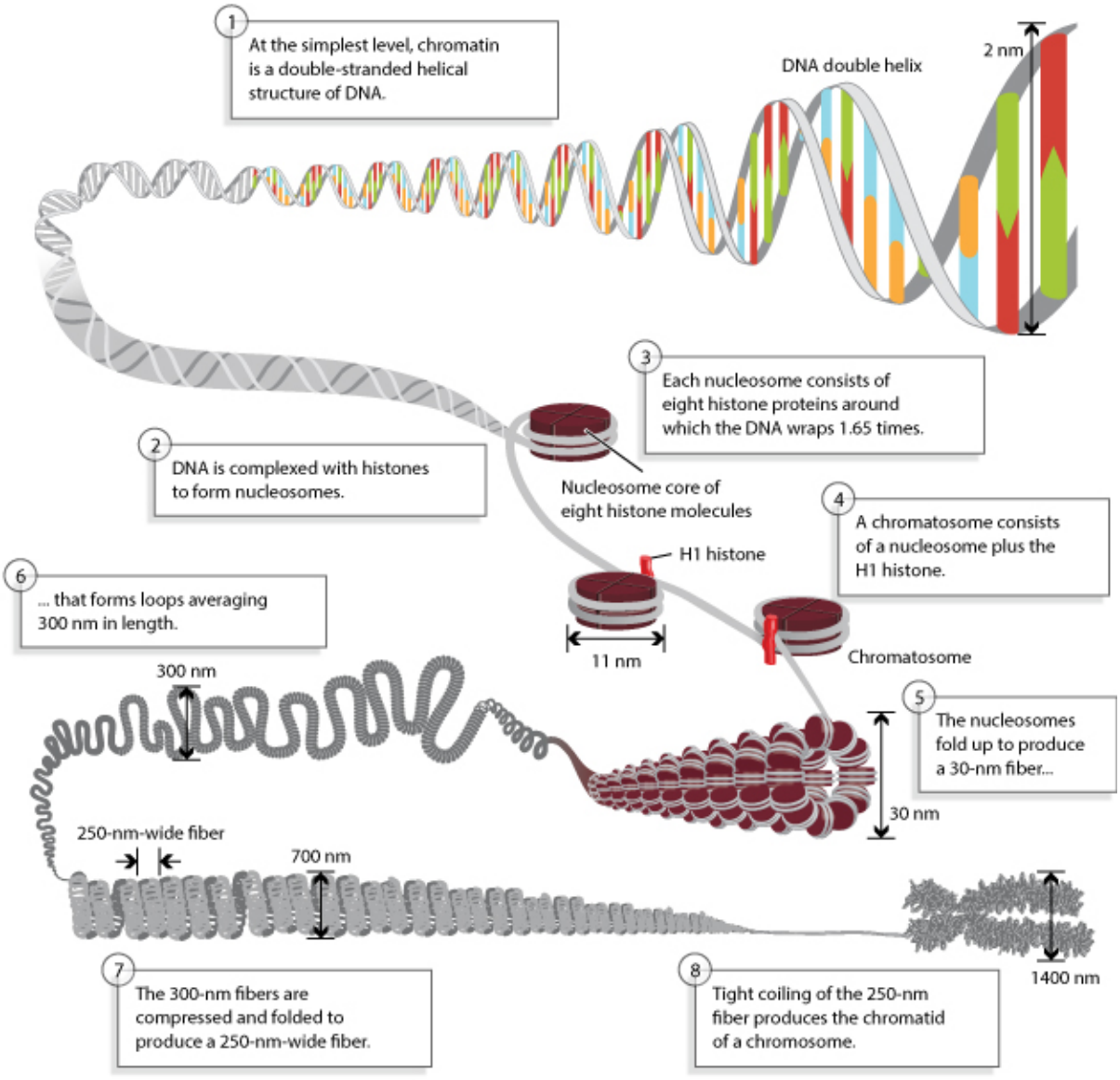
From Father to Son: Inheriting Fear

Can we inherit memories? In 2014, Brian Dias and Kerry Ressler conducted an experiment to test this. They exposed male mice to an aromatic compound (acetophenone) whose receptor gene (Olfr151) had been sequenced. This was followed by a non-lethal electric shock. This is pavlovian fear conditioning: instead of dogs drooling, you have rats cowering. They then took these scared mice and bred them with healthy, unconditioned females. They took the male offspring and subjected them to the same conditioning as the father.

What they found was that children of the conditioned father were more easily scared of acetophenone than any other smell. When they checked the mouse brain they found overexpression of the Olfr151 receptor, the protein required to "smell" acetophenone. Finally, though they found no genetic variation between litter mates, the authors found epigenetic changes in the Olfr151 gene of extra fearful siblings. It was less tightly coiled. They repeated all the above steps, looking at the grand and great grand children of the original mouse but the results did not change. The conclusion was hard to deny; memories are inheritable and over multiple generations.

The mice were not born with a blank slate. The shadow of their father's experience was cast over them. In humans, we have characterised this effect in families struck by famine, war and disease. Our ancestors tales are part of us. Not spiritually, but epigenetically in the spools of our DNA. Who our parents were is more a part of us then we could know. Epigenetics is then the bridge between nature and nurture. A resolution of Lamarck and Darwin and very likely the future of specialized medicine.

Genetically Engineered Mice
$50 with custom genetic code from JAX Pharmaceuticals



DNA Structure Explained

CRISPR and Gene Regulation

If you're into the biosciences, you've heard about CRISPR. **C**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats (CRISPR) and its Cas9 nucleases were discovered as part of bacterial immune systems in the 1970s; only in 2012 were they refined into a powerful gene-editing technology.

CRISPR/Cas9 is the new kid on the block, in company with a small list of gene editing techniques such as zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENS) which have been around since 1998 and 2009, respectively. What makes CRISPR special is that it's more highly targeted, cheaper, and computer-friendly. You can order a guide RNA (gRNA), the crucial code snippet for gene targeting, online.

While the technology works well for unicellular bacteria, it is not very effective in complex mammalian cells. By tweaking the software and hardware of this technology, scientists hope to make gains in controlled gene-editing.

Bacterial and Human Cells

Long before complex multi-cellular organisms like humans were in the picture, single-celled bacteria were our planet's dominant form of life. These bacteria competed and formed alliances with each other, and fought to protect themselves from viral invaders 1/100th their size - and bacteria are microscopic to begin with.

These viral invaders are short snippets of genetic material that cannot replicate on their own. They need a host to survive, so they sneak through the cell walls of bacteria and inject themselves into its genetic code, creating more of itself. Pretty smart.

CRISPR came from nature's own toolbox. Eventually, bacteria developed an immune system. It could absorb and remember the invader's genetic code, so that the next time the virus came around, the bacteria could send a nuclease after it, cutting the virus into useless bits. Even smarter.

CRISPR Trick #1: Cut-and-Paste DNA

"It's easy to reprogram [CRISPR/Cas] to go anywhere you want," says Oliver Medvedik, director of the Maurice Kanbar Center for Biomedical Research in New York City. Using a 20 nucleotide gRNA, the CRISPR system can easily target and lock onto a specific portion of our 3-billion-nucleotide-long genome.

Once locked on, it chops the DNA. Since cut-up DNA is about as useful as a cut-up tight rope, the body immediately works to repair the break. Human and mammal cells use two techniques for repair: non-homologous end joining (NHEJ) and homology directed repair (HDR). With CRISPR/Cas9, scientists can deliver a homology, or gene sequence, to insert into the cut site.

Since cut-up DNA is about as useful as a cut-up tight rope, the body immediately works to repair the break.

NHEJ is the default solution but is highly error-prone. The DNA repair systems quickly stick the DNA back together with just about any nucleotides they can find that fit. Usually this knocks out the gene by making it unreadable. In the worse case, it results in a functional mutation that is harmful to the host organism.

HDR is the preferred solution but occurs with less than 1% efficiency in human stem cells, for example, compared to 95% efficiency in unicellular yeast cells. Medvedik is working on methods to repress the NHEJ response and tweak the Cas enzyme for more efficient HDR repair.

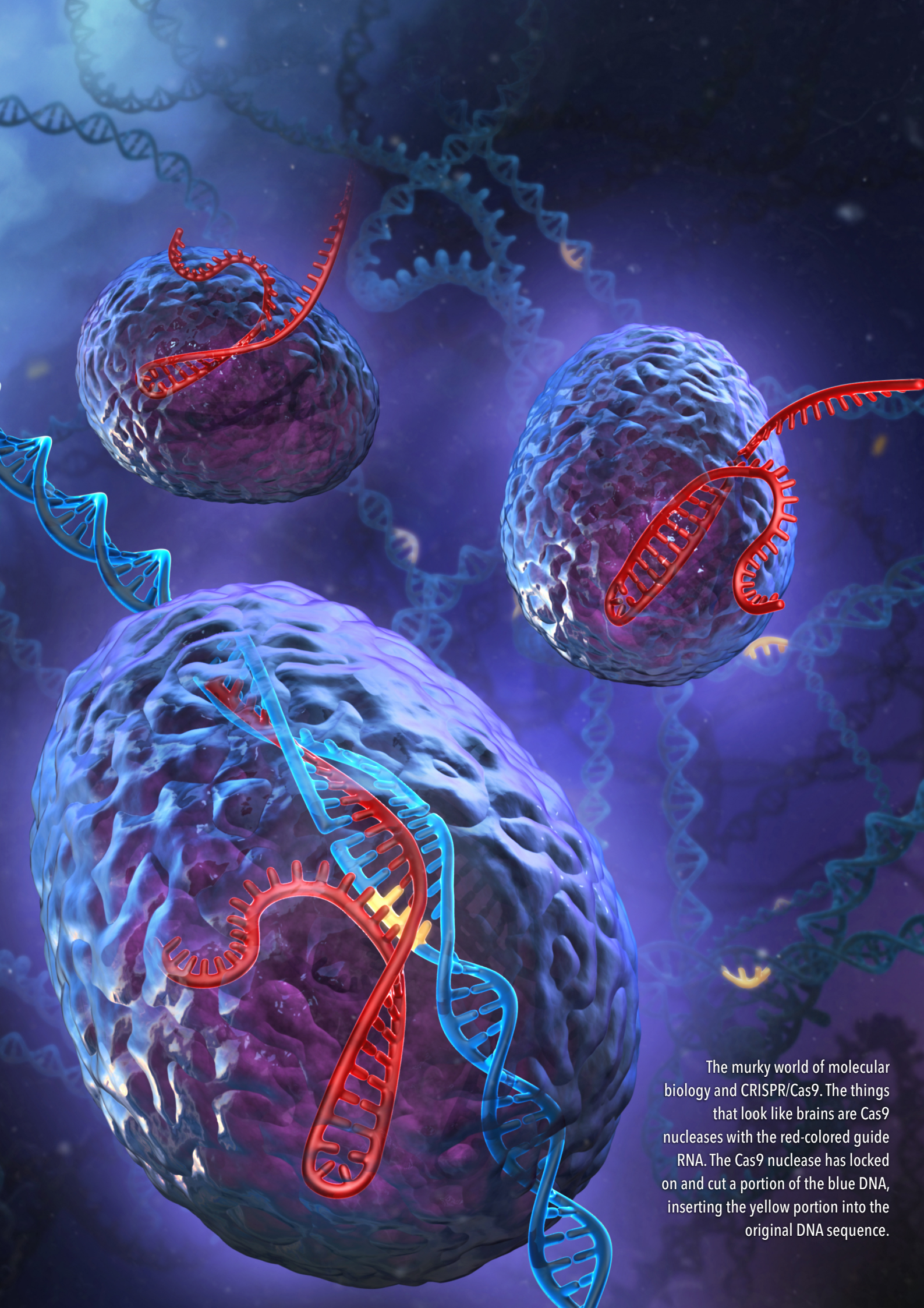
CRISPR Trick #2: Turn Off Disease Like a Light Switch

Scientists discovered that certain variations of the Cas nuclease, in combination with inhibitor proteins, could sit and lock on to DNA, silencing a selected gene. Modified versions go the other way, and remove inhibitor proteins, turning the genes back on. The technique, referred to as CRISPR interference (CRISPRi), is 95% effective in silencing genes whereas cutting them using Cas9 resulted in only 60-70% suppression and introduced greater risk.

CRISPR and Computers: Meatspace and Cyberspace

Previous gene-editing techniques required customized proteins for each gene sequence, an expensive and error-prone process. For CRISPR, changing a 20-nucleotide gRNA sequence is "ridiculously simple" and can be done online, says Medvedik.

He caveats, though, that "meatspace is different from cyberspace." Computers have drastically lower error rates and can run experiments in seconds, while bioengineering techniques run at high error with many months between the question and answer parts of the experiment.



The murky world of molecular biology and CRISPR/Cas9. The things that look like brains are Cas9 nucleases with the red-colored guide RNA. The Cas9 nuclease has locked on and cut a portion of the blue DNA, inserting the yellow portion into the original DNA sequence.

CRISPR: A Billion-Dollar Pie, But Not the Only One

CRISPR sits in the middle of a costly patent brawl. That hasn't stopped scientists and venture capitalists from betting on who will win. Business is moving fast with licenses being sold, awaiting the USPTO's decision in November which may declare a winner on the patent.

When it comes to pharmaceuticals, patents are licensed and traded in the amount of millions of dollars funneled through a 14 to 20-year timer.

While the stakes are high, CRISPR/Cas9 is not the only gene editing technology out there. It was however, the first gene editing technology to be discovered in ancient bacteria, channeling new interest with the hope to discover an unclaimed and more effective gene editing technique.

Who's Who in the CRISPR Patent Brawl

There are over 20 patents for various aspects of CRISPR technology, the patents are claimed and divvied up between four major companies: Editas with Zhang and MIT (public company, raised $190M), Caribou with Doudna and UC Berkeley (private, ~$42M), Intellia (public, $104M), and CRISPR Therapeutics with Charpentier (private, $154M).

While Doudna and Charpentier are largely touted as first to invent the technology, Zhang was first to file, with MIT paying extra fees for expedited filing services. Filed at the cusp between "first to invent" and "first to file" the patents hang in limbo until the next USPTO hearing in November.

At the heart of the dispute is the claim that Doudna only applied the patent for bacterial cells - and not for human cells, which are more complicated versions of bacterial cells. The dispute over what is and isn't obvious, along with timing of filing and evidence of first to discover, could point the patent, and millions of dollars of royalties, to either Doudna or Zhang.

Gene Therapeutics: Not Yet Approved, So What Now?

Pharmaceutical companies make money in two ways: drug sales and royalties on intellectual property. Since gene therapeutics are so new and governments haven't yet decided how to regulate the therapies, there are currently no sales in the U.S. and will likely be no sales for several years. So how do companies based off of CRISPR/Cas9 attract investment and operate?

Investors are betting that gene therapeutics will be profitable, and patents for CRISPR/Cas9 are held until 2033. Usually companies receive single-digit percentage royalties as well as yearly licensing fees on the scale of several hundred thousand dollars. If Editas wins the license, they can passively collect revenue on any drug sales from therapeutics using the Cas9 technology.

These pre-revenue companies also do collaboration with other large pharmaceuticals. Competing in the growing cancer space with chimeric antigen receptor (CAR) T-cells, Editas partnered with Juno Therapeutics receiving an upfront payment of $25M, while Intellia (founded by Caribou) has engaged in a collaboration with Novartis receiving an upfront payment of $10M. Entering the field of inborn metabolic disorders Intellia collaborated with Regeneron in April, receiving $75M upfront.

But Is CRISPR/Cas9 Actually That Big of a Deal?

While there is a Nobel prize floating around, and upfront license payments and collaborations are over $100M with billions to be gained in the coming 18 years, it also might not be that big of a deal in everyday research.

Zhang recently discovered another method in the CRISPR system called Cpf1, which staggers the DNA break and only needs one guide RNA. While the discovery is not revolutionary scientifically, being just one of many in the CRISPR system, and with many others bound to be discovered in the coming years, it does however provide an answer to the patent brawl by simply stepping out of it. Cpf1 can do the same thing, without all the legal hassle. And there's more where it came from.

Zhang recently discovered another method in the CRISPR system called Cpf1

How does all this affect day-to-day science? Speaking with Alex Chavez with the Church Lab, who is researching modified CRISPR/Cas9 systems to treat Duchenne's Muscular Dystrophy (DMD), I ask if the CRISPR patent brawl will affect scientists' choice in research. Luckily he responded, "I think a lot of people are driven purely by intellectual curiosity, and if [money] comes along, that's great."

Jennifer Doudna heads the Doudna lab at University of California San Diego. She founded Caribou Biosciences which is working closely with Intellia.

Feng Zhang is one of 8 core faculty at the Broad Institute. He recently discovered Cpf1 and co-founded Editas Medicine, based in Cambridge, Massachusetts.

Emmanuel Charpentier and Jennifer Doudna received the Breakthrough Prize in Life Sciences in November 2014. She founded CRISPR Therapeutics in Basel, Switzerland with R&D in Cambridge, Massachusetts in 2013.