NAUTILUS

BIOLOGY + BEYOND

SPECIAL CSHL ISSUE | FALL 2022

Nautilus is a magazine of scientific ideas that matter, featuring stories that will challenge and enrich the way you view the world. Discover new, surprising perspectives on how science interacts with all aspects of life—medicine and health, of course—but also politics, art, food, work, technology, and sports.

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EDITOR'S NOTE

Our Infinite Potential

BY LINA ZELDOVICH

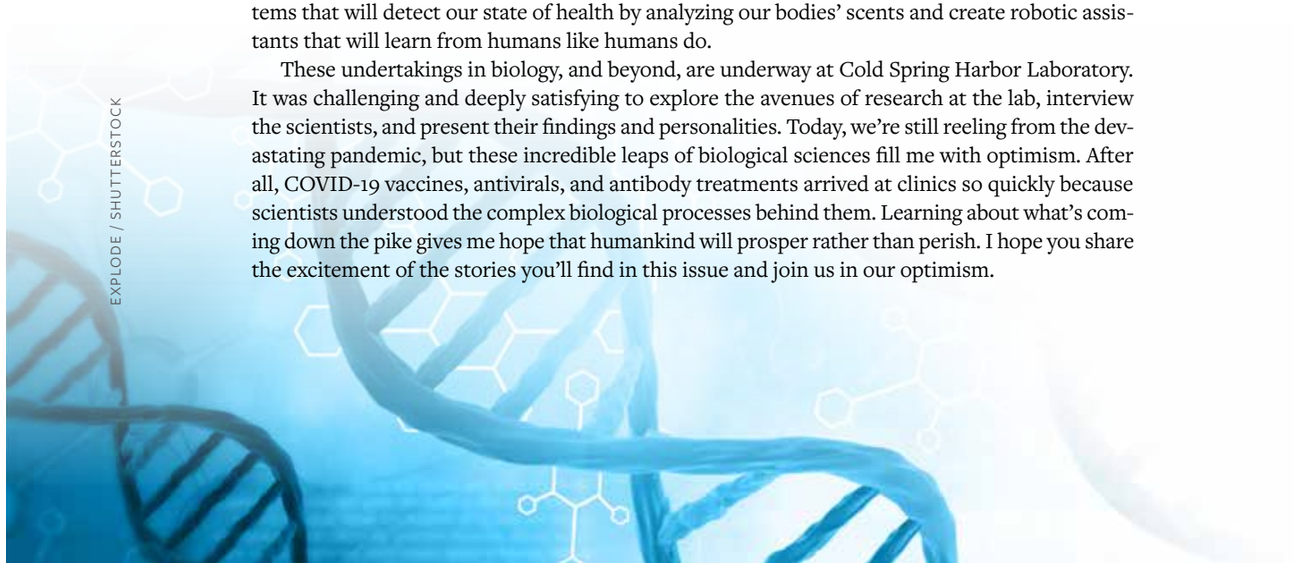
WHEN I FIRST BEGAN writing and editing stories for Biology + Beyond, a special section on *Nautilus*, created in partnership with Cold Spring Harbor Laboratory, I was both humbled and inspired. I was humbled and inspired by current research at the lab, founded in 1890, and home since then to eight Nobel laureates. But I was also humbled and inspired by the discipline of biology itself. Its potential for improving our world is limitless.

One of the oldest scholarly subjects, biology is a foundation for many modern scientific fields. Without biology, there would be no medicine. Pharmaceutical research likely wouldn't exist either. Even botany relies on the biological activities happening within plants' cells. With the invention of a powerful microscope in the 17th century—thank you, Antonie van Leeuwenhoek—microbiologists went on to discover numerous pathogenic microbes that plagued humankind with infectious diseases. That brought the advent of modern medicines—antibiotics, vaccines, and other therapeutics that saved, and continue to save, millions of lives.

High-resolution electronic microscopy and genome sequencing tools enabled scientists to do much more. Researchers can eavesdrop on cellular communications and intercept the messages that cells send to each other. Scientists can peek deep inside the cells and watch the complex interplay of DNA and the RNAs, which carry out the myriad vital functions without which no organism could last a day. Microbiologists can see how malignant cells dodge the immune system's defenses and hijack the body's nourishment to feed themselves.

That plethora of knowledge allowed scientists to ponder—and in some cases already bring to market—the new generation of medicines which kill cancers in novel ways, repair previously incurable genetic conditions, and stimulate the immune system to fight off diseases on its own. Some of these advancements will also help evolve stronger, healthier plants, ensuring a secure food future for humankind. Others will allow scientists to build smart artificial intelligence systems that will detect our state of health by analyzing our bodies' scents and create robotic assistants that will learn from humans like humans do.

These undertakings in biology, and beyond, are underway at Cold Spring Harbor Laboratory. It was challenging and deeply satisfying to explore the avenues of research at the lab, interview the scientists, and present their findings and personalities. Today, we're still reeling from the devastating pandemic, but these incredible leaps of biological sciences fill me with optimism. After all, COVID-19 vaccines, antivirals, and antibody treatments arrived at clinics so quickly because scientists understood the complex biological processes behind them. Learning about what's coming down the pike gives me hope that humankind will prosper rather than perish. I hope you share the excitement of the stories you'll find in this issue and join us in our optimism.



EXPLODE / SHUTTERSTOCK

Welcome to Biology + Beyond

WELCOME TO A SPECIAL print edition of Biology + Beyond, a storytelling collaboration between Cold Spring Harbor Laboratory (CSHL) and *Nautilus* that invites you to discover the many ways biological research impacts our lives.

We're very excited about the wide range of topics in this issue, which showcase the diversity of CSHL's research. You're guaranteed to find a story that speaks to your own curiosity.

For example, did you know that mothers are genetically wired to nurture their young? Or that next generation artificial intelligence depends on neuroscience? Did you think growing tissue samples in 3-D environments might make a difference for pancreas cancer patients? Have you wondered how much genomic knowledge goes into a single ear of sweet corn? Or how many ancient viruses are lurking in your own genome?

CSHL is the place where this range of questions come together, because of our unique perspective on biology. Our researchers are curiosity-driven and are encouraged to collaborate across multiple disciplines to explore what is unknown.

We are a private, not-for-profit institution continuously evolving the fields of molecular biology and genetics through research initiatives, pioneering science education, and innovative platforms for science communication.

Discover CSHL through the stories in this magazine. Join our journey of biological discovery by participating in education and public outreach programs at CSHL.EDU.

—Bruce Stillman
President & CEO
Cold Spring Harbor Laboratory

Preludes

TOROOK / SHUTTERSTOCK

NEUROSCIENCE

Unlocking Mom's Brain

LIFE ISN'T ALWAYS EASY for little mouse pups: Hours to days after they are born, the squirmy babies, who can't hear or see, can roll or stumble away from their nest. Cold and lonely, they call out to their mother. Luckily, Mom snaps into action to ensure the adventures of the little ones are short-lived. Grabbing each pup by the skin on their backs, Mama mouse brings each baby back home to safety.

The mom's behavior is innate, burnt into the mouse brain, and requires no training. But where in the brain does it happen and how does the brain process or execute it? And what happens in those rare cases when the animal brain doesn't properly execute such behavior? That's what Stephen Shea is trying to answer in mice, with hopes that it may someday be applicable to humans.

Shea, an associate professor at Cold Spring Harbor Laboratory, discovered that this innate mothering behavior corresponds to the firing of cells in a region of the brain called locus coeruleus, a cluster of cells that can be found in the brainstem of all vertebrates. Locus coeruleus is also the source of noradrenaline, a chemical that affects some key brain functions.

Shea's work has greater implications. He hopes that understanding the brain circuits that facilitate this very simple action could be a window into how disruptions in wiring affect social behavior, and a key into understanding inappropriate social interactions, such as those

Shea's work may shed some light on whether creatures are shaped by nature or nurture.

observed in people with autism spectrum disorders. And it may even shed some light on the iconic debate about whether creatures are shaped by nature or nurture.

When social animals encounter one another, they interact by taking in sensory inputs (such as sounds and smells), processing them in the brain, and responding appropriately. In popular conception, there are two competing factors that determine animal or human behavior, hard wiring and learned traits, or as it's commonly known nature versus nurture. Neuroscientists and biologists, however, don't think of animal responses as being the product of one or the other but an interplay between the two. One of the ways to understand this back-and-forth in the brain is by working backward, through the lens of behaviors that can be innately expressed, such as the maternal ones in mice, and then observing how the innate expression of that behavior is further modulated by learning.

"We study social communication behavior between mice," Shea says. "Mice talk to one another, they smell one another, and they can learn a lot from that. We want to understand that process, how that's represented in the brain [and] what the mechanistic controls of those behaviors are."

Though innate in the neural representation of maternal behavior, mice change their behavior toward the pups under different conditions through plasticity in the brain. For example, virgin mice tend not to actually show such caring behavior, ignoring or even killing the young in some cases. But if the females are co-housed with a mom or repeatedly exposed to the pups, they act like moms themselves and their behavior changes. They start to care for the pups and become sensitive to their cues so they will rush to the baby mice when they wander away from the nest.

"How that happens in the brain is not well understood," says Dayu Lin, a professor at the Department of Neuroscience and Physiology and Department of Psychiatry at NYU Langone Health who is not involved with Shea's research. "Something must have changed in the brain to cause the animal to show different kinds of behaviors toward the pup, so Dr. Shea's effort is mainly trying to understand which part of the brain is relevant to what process and what kind of changes have happened in those parts of the brain."

Over the several years that Shea has been working on this, he has made some important discoveries. In addition to finding the region that corresponds to the behavior, he found that animals that carry a mutant copy of a protein called MeCP2 have difficulty learning such maternal retrieval behavior.

"The brain becomes more fixed and doesn't have the capacity to change," Lin says. "And that is one of the reasons why animals are unable to learn those innate behaviors."

When mutated, MeCP2 is also responsible for a neurodevelopmental disorder called Rett syndrome in humans, which afflicts primarily females, affecting their ability to speak, walk, eat, and even breathe easily. If researchers can understand how MeCP2 affects the mental or developmental ability to acquire and maintain communication circuitry in mouse models, they might pick up clues as to how it may cause problems in humans.

"MeCP2 causes a fundamental disorder because it plays an important role in the brain, and we think it's important for controlling plasticity, which is the ability of the brain to change and adapt to new conditions, and one of those conditions is actually maternity," says Shea. He explains that when a healthy female mouse has a maternal experience, either because she has her own pups or because she encounters the pups of another female, there are changes in the auditory cortex, the part of the brain that encodes or detects auditory stimuli, including the pups' distressed squeaks and other vocalizations. Understanding how the brain can change in health and disease increases our fundamental knowledge of how it wires and rewires itself and what could go wrong.

To study the mice, Shea and his colleagues utilize optical methods to look at the brain's electrical signals. In the brain, axons, which carry information from one area to another, do so through electrical impulses, acting a bit like wires. Researchers use a virus to deliver a genetic piece of DNA to some neurons and make those neurons produce a protein that is a sensor for activity. What they detect is the so-called calcium signals that are correlated with electrical activity. When calcium levels rise in the cell, the protein glows brighter, allowing researchers to see the activity of neurons.

Neurons, which talk to each other through synaptic communication, affect those they contact. So, excitatory neurons can activate the neurons that they give input onto, and inhibitory neurons suppress the activity of the neurons they give input onto.

In many ways, these two neuron types work like a seesaw. Both are needed for control of stable neuron activity patterns, and "one of the ways the brain can change is it can tip that balance away from inhibition and toward excitation," Shea says. "So, when a mouse has a maternal experience, there's a disinhibition of the brain. That tips that seesaw more toward excitatory and away from inhibitory and that is a feature that defines this critical period, and that balance is not maintained properly in the MeCP2 mice."

Shea would like to get as close as possible to a working model of interactive behavior that goes from detection of a signal at the periphery to how that signal is processed, weighed against other factors in the decision-making process and how an animal chooses a behavior as a full loop. And lastly, how that affects the behavior of a social partner.

"So, what I'd like to be able to do is understand the brains of two or more individuals and their behavior as a dynamical system," he says.

Such a dynamical model would help researchers understand the decision-making process, along with the ability to make predictions about changes in the brain. So, if the activity in one part of the chain is perturbed, they can observe how that would affect the individual and their social partners.

"In the context of innate behaviors, which are presumed to be relatively hardwired inside of a brain, you still see a huge amount of changes that happen," says Lin. "So, I think [this work] really helps us in understanding the flexibility capacity the brain has and how much we can move to guide different behavior output." In more layman terms, Shea's work speaks to that centuries-old nature-versus-nurture debate. Creatures, whether animals or humans, aren't shaped by either one or the other. We are the product of both.

—Sara Goudarzi

ENVIRONMENT

The Environmental Headache in Your Shampoo

AS LITTLE AS two centuries ago, the northern edge of the island of Borneo, home to Malaysia's Sarawak state, was covered in a verdant canopy that stretched, uninterrupted, from shore to shore. It was a forest that had persisted for more than 100 million years, sheltering a dizzying abundance of plants, animals, and fungi that were found nowhere else on Earth. It survived the extinction of the dinosaurs and countless cycles of glaciation. It housed humans for 40,000 years while our species grew and grew around the world.

Then, over the past few decades, the forests of Sarawak faced threats unlike any before. The canopy began to recoil, its edges assaulted by the expansion of hydroelectric power, logging, and, most impactful of all, palm oil plantations. To many people, these changes look like the necessary costs of progress. Development has consumed almost a third of the forest, but it has also lifted millions out of poverty. The first wave of palm oil plantations, from the 1970s to the 1990s, provided farmers with seven times the income of subsistence-food croppers in the same

TAFILAH MD YUSOF/POND5

regions. Industry has brought paved roads, better schools, and modern information infrastructure.

The oil palm (formally *Elaeis guineensis*) is a Shiva of the modern consumer economy, a great creator and a great destroyer. A startling amount of human happiness and well-being depends on our relationship with this one plant. Presently palm oil accounts for 60 percent of all cooking oil, more than 62 million tons in total. It's found in half of supermarket goods, from instant noodles to ice cream, air fresheners to shampoos. You may not see it but you are eating it and washing your hair with it. Consumer-product manufacturers prefer palm oil because it blends well with other oils and is the ideal elixir to create various consistencies. Savvy food marketers love it because it contains low levels of artery-clogging trans fats.

You'd have to look far and wide to find a major company that doesn't have palm oil on its hands. They include Walmart, Colgate-Palmolive, Kellogg's, Nestle, McDonalds, Ikea, Target, and Whole Foods. Palm oil is mixed into animal feed and biofuels.

Malaysia accounts for 26 percent of the vast production of palm oil today, making it a great creator for the local economy as well. Almost half of oil palms in that country are grown by smallholders rather than large-scale agribusiness. The crop is so important that government insiders consider its development synonymous with the eradication of poverty in Malaysia. Between 1980 and 2010, palm oil cultivation doubled in Malaysia. Then, in just four years, it doubled again.

Therein lies the seemingly intractable dilemma of humanity's intimate relationship with this tropical tree. Palm oil production is phenomenally important to local peoples and international economies. But it is also tremendously destructive to natural ecosystems and to the global climate.

The oil palm is a Shiva of the modern consumer economy, a great creator and a great destroyer.

Tropical forests and peatlands are great storehouses of carbon dioxide, the main gas indicted in global warming. Malaysia's forests are especially rich in carbon. They can hold up to 220 pounds of carbon per square mile. "That's equivalent to the emissions from driving an average car from New York to San Francisco and back 76 times," the Union for Concerned Scientists tells us. Razing forests and peatlands unleashes carbon dioxide into the atmosphere in calamitous amounts. Deforestation for palm cultivation in Indonesia accounted for 2 to 9 percent of all tropical land use emissions from 2000 to 2010. Palm oil expansion is also robbing orangutans, tigers, rhinos, and elephants of their natural habitats. Global demand for palm oil is expected to increase from 76 million tons in 2019 to over 400 million tons in 2050.

Environmentalists are realists enough to know that palm oil is here to stay. Too much money and too many powerful government and community interests are tied up in its production. You don't overturn the world economy overnight. Plenty of nonprofits are pushing sustainable harvesting of palm oil and an international movement, Roundtable on Sustainable Palm Oil, is signing up companies to pledge to employ smart environmental practices. These grassroots efforts, though important, may amount to little more than a superficial solution, however.

What's most needed is way to reboot our relationship with the oil palm—to find a way to produce more oil on less land. Here is where plant scientists must step in. And they have. They have crafted a novel genetic technique to induce each palm oil tree to produce more fruit, containing more of the precious oil. It's a way to keep the ice-cream makers happy while saving the rainforest, and it can be scaled up now.

ROB MARTIENSSEN, A PLANT BIOLOGIST at Cold Spring Harbor Laboratory, has been one of the world's key researchers into the puzzles of palm oil production: why scientific methods have gone wrong in the past, and how to right those wrongs today. Launching a project to grow more palm oil on less land was the easy part, he knew. Scientists locked in on that goal some decades ago and set out to clone a single "elite" palm, one that produced a bounty of oil, into 50,000 palms just like it. They even succeeded, up to a point. "They thought this was going to solve all problems," Martienssen says, but cloning the elite palm in the lab turned out to offend the plant's natural growth processes. Once planted, the identical trees were "mantled": Instead of yielding the promised bounty, the plants produced gnarled fruit that gave no oil.

It took an international collaboration between Martienssen's group at Cold Spring Harbor Laboratory and their colleagues at the Malaysian Palm Oil Board nearly 20 years to unravel the mystery of the mantled fruit. They started by assembling and analyzing the whole genome sequence of the *Elaeis guineensis* oil palm. That enormous effort only made the problem more puzzling, however, because they found no genetic differences between normal and mantled clones.

Rather than give up, the researchers dove even deeper, beyond the DNA of the oil palm and into the layer of biology that regulates how DNA is read and translated: the epigenome. To their astonishment, they found that the huge difference in the mantled clones was the result of a single, tiny epigenetic change. Palms that produce mangled fruit have an altered molecular switch that interferes with expression levels of genes relevant to healthy fruit production. Previously that miscreant switch had been identified in rice plants and was named "karma." The palm clones literally suffered from bad karma.

"In terms of individual palms, if you have bad karma, then it's going to literally get no oil," Martienssen says. With the mechanism behind mantling unmasked, a third partner—Orion Genomics, a private startup founded by Martienssen—was able to develop a simple DNA test that predicts whether a designer seedling will bear robust or withered fruit. Then only the genuine, high-yield clones will make their way into the field.

That epigenetic test could be making a difference in the oil-palm plantations very soon. "It's currently being commercialized jointly by the Malaysian Palm Oil Board and Orion Genomics," Martienssen says. He projects that reliable clonal stocks could increase yields by 30 to 50 percent, drastically reducing the pressure for illegal forest clearing. And that's just the start. Other scientists are working on dwarf varieties of the oil palm that are easier to harvest, that come to maturity faster, and that stay in production for longer. The epigenetic test can be applied to genetically modified palm varieties for a synergistic effect, but—important for many consumers and environmentalists—it provides major benefits on the non-GMO clones as well.

In Malaysia, the government is finally acting to protect what's left of Sarawak's ancient forest canopy. New policies limit the expansion of palm plantations to 6.5 million hectares, which leaves just 1 million more hectares of land for cultivation. These moves create strong incentives to enact a better, smarter relationship between humans and the plants they rely on. "From a world production point of view, palm oil is not going away," Martienssen says. "Reducing its footprint is the best thing we can do to help the rainforest."

—Anastasia Bendebury & Michael Shilo DeLay

MICROBIOLOGY

The Non-Human Living Inside of You

Eight percent of our DNA consists of remnants of ancient viruses.

THE HUMAN GENOME contains billions of pieces of information and around 22,000 genes, but not all of it is, strictly speaking, *human*. Eight percent of our DNA consists of remnants of ancient viruses, and another 40 percent is made up of repetitive strings of genetic letters that is also thought to have a viral origin. Those extensive viral regions are much more than evolutionary relics: They may be deeply involved with a wide range of diseases including multiple sclerosis, hemophilia, and amyotrophic lateral sclerosis (ALS), along with certain types of dementia and cancer.

For many years, biologists had little understanding of how that connection worked—so little that they came to refer to the viral part of our DNA as dark matter within the genome. "They just meant they didn't know what it was or what it did," explains Molly Gale Hammell, an associate professor at Cold Spring Harbor Laboratory. It became evident that the virus-related sections of the genetic code do not participate in the normal construction and regulation of the body. But in that case, how do they contribute to disease?

An early clue came from the pioneering geneticist Barbara McClintock, who spent much of her career at CSHL. In the 1940s, long before the decoding of the human genome, she realized that some stretches of our DNA behave like infectious invaders. These DNA chunks can move around through the genome, copying and pasting themselves wherever they see fit, which inspired McClintock to call them "jumping genes." Her once-controversial idea earned her a Nobel Prize in 1983.

ILLUSTRATION BY KATERYNA KON

Geneticists have since determined that jumping genes originate in the viral portion of the genome. Many of these genes turn out to be benign or even helpful. “But some of the things are full-on parasites,” Hammell says, like infections embedded within our own DNA. All it takes to set these bad actors loose, she is finding, is a slip-up in the body’s mechanisms that normally prevent the genes from jumping around and causing harm.

Much of the research on the connection between jumping genes and disease has focused on natural molecules in the body that immobilize the genes by blocking their sequences from being read or copied. In recent years, Hammell and a number of scientists have focused specifically on a once-obscure protein known as TDP-43, which is highly adept at latching onto and hiding stretches of DNA.

Avi Nath, the clinical director of the National Institute for Neurological Disease and Stroke, helped draw attention to the importance of TDP-43 starting a decade ago. While studying a group of HIV-positive patients with ALS-like symptoms, Nath found that the anti-HIV drugs they were taking were also improving their ALS symptoms. He suspected that the drugs designed to fight the HIV virus were also suppressing the virus-like activity from jumping genes.

Subsequent work by Nath and others bolstered that idea, identifying a specific group of viral relics that seemed to be associated with dead neurons in the brains of ALS patients. A study led by biochemist Wenxue Li, now at Yale University, further showed that the ancient viruses in question interact strongly with TDP-43.

At this point, the puzzle pieces began to fall into place. Medical researchers already knew that nearly all ALS patients experience a severe TDP-43 malfunction that causes large amounts of that protein to build up in their neurons, where it forms toxic clumps. Now it appears that TDP-43 could contribute to ALS in another way: The faulty form of the protein might no longer be able to hold back critical nerve-killing jumping genes.

Hammell has confirmed that the normal form of TDP-43 suppresses harmful activity from jumping genes in mice and humans. Other researchers have found that the TDP-43 malfunction is also associated with certain types of Alzheimer’s and dementia.

The case is still not completely solved. Hammell and Nath cannot yet say for certain whether jumping genes cause ALS in some patients, or whether their activity is a byproduct of the way that ALS progresses. But either way, researchers have an important new goal in treating neurodegenerative disease: taming the non-human portion of our genome.

—Carrie Arnold

Beautiful Brain

SHL PROFESSOR ANTHONY ZADOR has developed mapping technologies that identify connections of individual brain cells, pinpoint where a cell is located, and determine the specific function of that cell. Detailed wiring diagrams—connectomes—for the brain are critical for understanding brain development, function, and disease.

These technologies, called MAPseq and BARseq, use genetic barcodes, or short sequences of DNA and RNA, to trace thousands of brain circuits simultaneously. This approach increases the number of neuronal connections that can be traced at a time versus traditional microscopy-based methods that rely on fluorescent markers or dyes to trace neuronal activity. Zador's genome sequencing technology approach allows researchers to label many neurons and brain regions at once.

MAPseq labels each neuron with a unique genetic barcode so that researchers can determine where a neuron projects by dissecting brain regions of interest and searching for the barcode. BARseq is the next generation of MAPseq, allowing barcodes to be sequenced within a tissue, providing a method for in situ sequencing in neurons. This approach preserves anatomical information and allows scientists to examine cellular connectivity at greater resolution, which has several possible applications including cell typing and brain mapping. In this image, BARseq2 has been used to detect RNA from dozens of genes in thousands of neurons within one section of a mouse brain. Each color lights up a different set of genes.

Image credit : Xiaoyin Chen & Yu-Chi Sun | Zador lab

DISCOVER CSHL

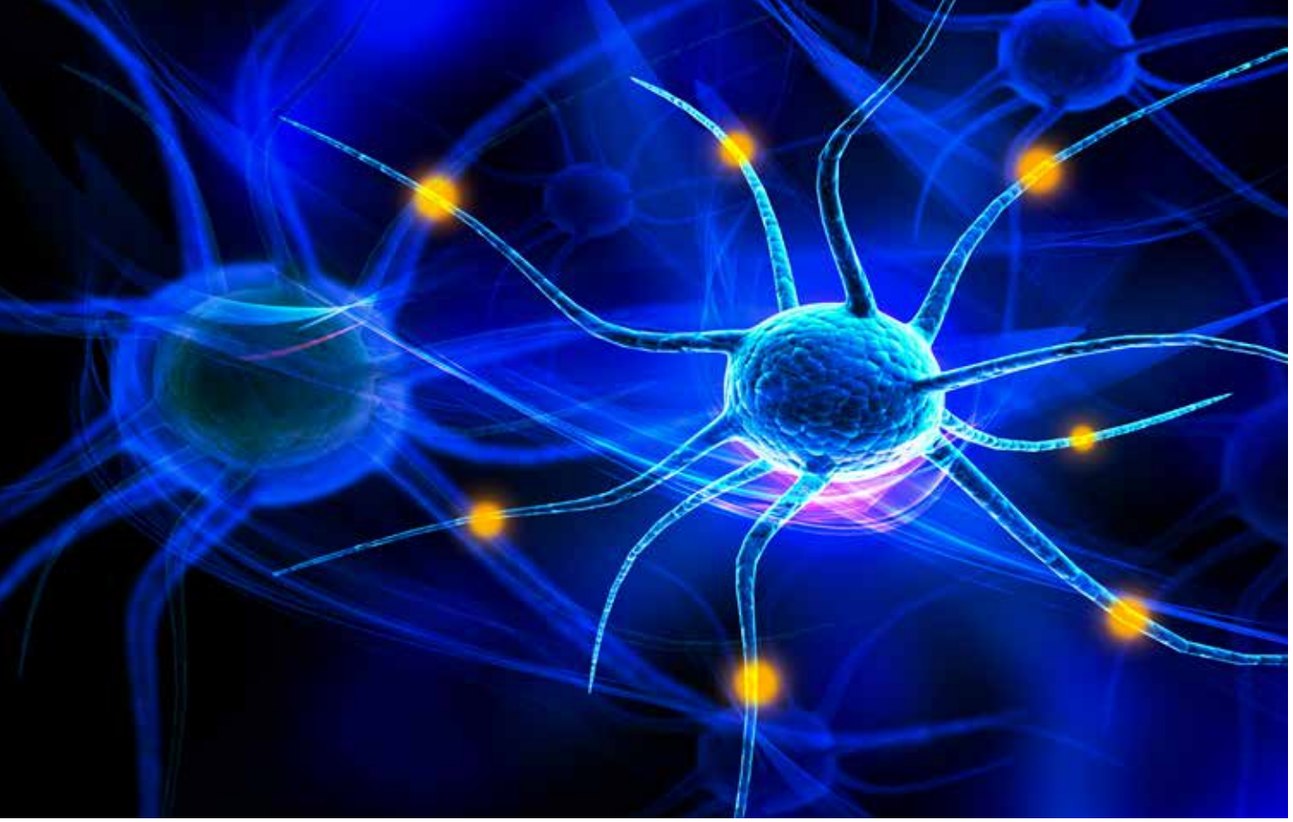
Cold Spring Harbor Laboratory

CSHL.EDU

FROM THE SHORES OF LONG ISLAND Sound and facilities across the New York metro area, CSHL has shaped contemporary biomedical research and education since 1890.

Home to eight Nobel Prize winners, the private, not-for-profit research institute is a global leader in cancer, neuroscience, plant biology and quantitative biology.

By design, our 1,000 employees—including 600 scientists, students and technicians—approach biology from unique perspectives.



RENJITHKRISHNA / SHUTTERSTOCK

Cancer's Got a Lot of Nerve

Tumors recruit the nervous system to help them spread. Scientists are looking for ways to stop it.

BY LINA ZELDOVICH

MANISH VIRA, A UROLOGIST at Northwell Health in New York performs prostate biopsy procedures three to five times a week. He inserts 12 needles into specific locations on the prostate gland, identified by MRI images that reveal malignant or suspicious lesions. The samples then go to a pathologist who determines whether cancer is present and how aggressive it is. "It's a standard protocol," explains Vira, who is also a chief oncologist at Northwell.

For the past few years, however, that standard protocol has had a few extra steps. Now, the biopsy "wash"—a collection of molecules washed off the sample—goes to the research lab of Lloyd Trotman, a professor at Cold Spring Harbor Laboratory who studies what makes these tumors aggressive or aids their metastases. Trotman's team looks at the tumors' genomic signatures—their genetic makeup, which can make them more aggressive. They look at the tumors' microenvironments—the molecules that cancer surrounds itself with. And while researching these factors, they also dig into something that's rarely looked at in cancer biology: the nervous system and its role in helping tumors spread.

Tumors don't exist in a vacuum.

In the past decade, scientists have realized that cancer isn't just a localized disease, but a systemic problem that involves the whole organism. They realized that tumors don't exist in a vacuum but require a permissive and supportive environment to take hold and thrive. Tumors create their own ecosystems, in which they recruit and reprogram the body's own cells—sometimes even those that are supposed to destroy cancer—to help them grow and travel to new locales. What is less understood is the role that nerves play in these complex physiological interactions, says Jeremy Borniger, an assistant professor at CSHL Cancer Center, who works with Trotman. In the complicated scenario of how tumors burgeon and metastasize, the nervous system had not received its due attention.

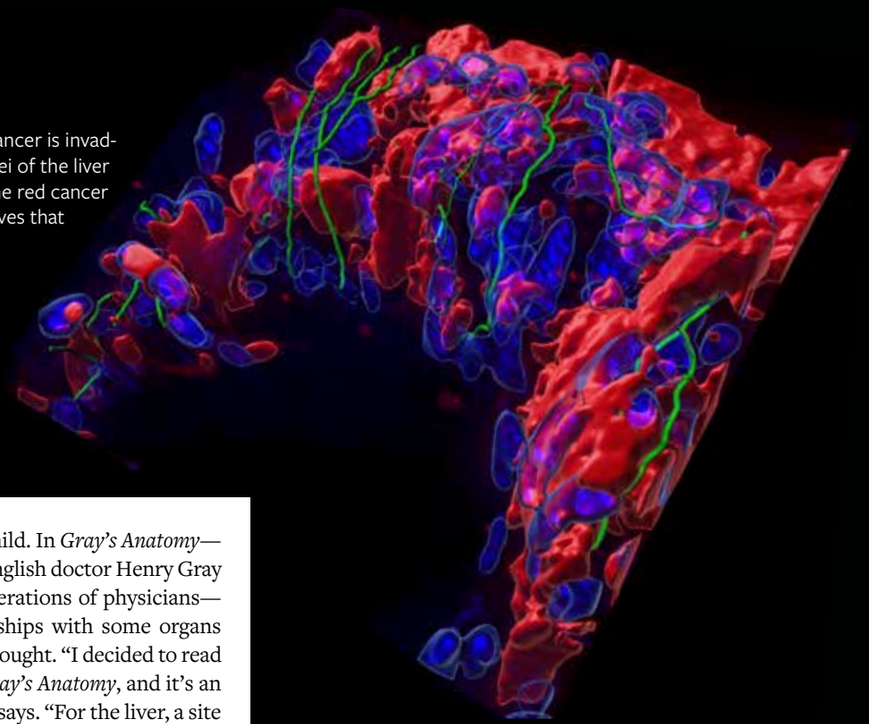
"If you look at any cancer review paper from until about five or six years ago, you see that the research has been hyper-focused on the oncogenes, the tumor microenvironment—cancer cells, immune cells, endothelial cells, fibroblasts, maybe a couple of other cell types," says Borniger. That biological picture is of course important, but not complete. "The nerves are almost never mentioned. So what's been missing from the equation is how the tumor interacts with the body on the physiological scale, such as with the nervous system."

Trotman and Borniger try to view cancer as a whole-body disease, in which the brain (the central processing unit) and the nervous system (its communication channels) occupy an important spot in cancer progression—perhaps even the final frontier in our understanding of this disease. For starters, no single organ can exist without being "innervated"—served and attended by nerves, which deliver signals back and forth from the brain, directly affecting how each body part functions. The brain, for its part, is the master regulator of the body, coordinating all chemical processes that happen inside us. The brain collects the information about the body through the nervous system and via circulating chemical cues in the blood. Then, it interprets the info and sends back chemical messages to neurons that pass them to the organs, muscles, and glands—to monitor and influence the activity of those tissues.

"The nervous system controls everything in normal tissues—growth or atrophy, or anything else," says Massimo Loda, a molecular pathologist at the Weill Cornell Medical Center in New York. So there's a reason to believe that the same is happening with malignancies. "Cancer tissue grows fast so it needs the support of the nervous system," Loda says. Moreover, scientists know that certain cancers have a particular predilection for nerves. "For example, breast and prostate tumors have a propensity to look for nerves and kind of invade and travel through those nerves," Loda says. It is as if there are some shadowy dealings happening between the nerve endings and tumors. "That suggests that there is synergy there."

The observational knowledge suggests that a greater amount of nerves bunching up around a tumor signals grimmer prognosis. For example, when pathologists assess the severity of prostate cancer, the number of nerves that surround these tissues factors in. "The pathologist will score that, and if there's a lot of nerves in the area, it usually means a worse, or a more urgent situation," Borniger explains. "To us, that seems like a blind spot or a missing link."

IT'S NOT FULLY CLEAR why the nerves and their involvement in cancer had languished in scientific obscurity for so long, but scientists have a few ideas. As strange as it sounds, the peripheral nervous system was



INVADING ARMY Prostate cancer is invading the liver. In blue are the nuclei of the liver cells that are being invaded by the red cancer cells. The green lines are the nerves that cancer may have used to spread.

COURTESY OF MARY DOHERTY OF CSHL

the anatomy textbooks' stepchild. In *Gray's Anatomy*—the medical bible written by English doctor Henry Gray in 1858 that still educates generations of physicians—the nerves and their relationships with some organs remain somewhat of an afterthought. "I decided to read the current 42nd edition of *Gray's Anatomy*, and it's an interesting picture," Trotman says. "For the liver, a site of end-stage prostate metastasis, you see descriptions and depictions of all kinds of cells, all kinds of conduits and blood vessels, but to this day the nerves are usually not depicted. The innervation of some organs is apparently not a major topic in the organ anatomy."

Modern science, of course, pays far more attention to the nervous system and the brain than the 150-year-old manual. In 2016, the Allen Institute for Brain Science published a map of the entire human brain—a digital atlas of our central processing unit. A 2021 effort preserved, sliced, and imaged a human surgical fragment of a cerebral cortex. And yet, the peripheral nervous system, which is the conduit between the brain and the rest of the body, is still not fully specced out. "We don't really have great maps of the peripheral nervous system, and how it connects the brain to these organs," Borniger says.

Another reason for this strange disconnect is that traditionally neuroscientists rarely talked to cancer biologists. "Neuroscientists don't typically work on cancer and cancer biologists typically don't work with neuroscience questions," Borniger says. Historically, the two disciplines remained too distant from each other and too siloed inside their own respective dominions.

"That's what we are trying to change," Trotman says—essentially merging the two fields to study the neuroscience of cancer. In that realm, the prostate makes a particularly good research subject, he explains. "Prostate is a gland, which means that it already has a lot of nerves surrounding it," he says. "The nervous system controls how the gland functions, such as squeezing out liquids. So it's already organized in a way that's amenable to our research." And with the new tools that became available to scientists in the past decade or two, they are now able to peek at those shadowy nerve-tumor dealings in real time.

Several major technology breakthroughs are making visualizing nerve and tumor interaction possible. One of them was the usage of fluorescent technologies that allowed scientists to engineer tissues to glow a certain color—red, green, blue. Another major advancement at peeking into the tangly webs of nerves, neurons, and axons were the optogenetic tools that let researchers manipulate the activity of neurons with light.

Neuroscientists rarely talked to cancer biologists.

With these technologies, mice can be engineered in such a way that whenever a tumor naturally grows in them, it fluoresces in, for instance, the color red. "That means that any descendants of those cancerous cells will also glow red wherever they go," Trotman explains, which gives researchers a way to see how metastases spread and take hold. Similarly, mice can be engineered to have organs innervated by nerves of shimmering green or some other hue. That gives researchers an unprecedented opportunity to see how nerves and tumors play together. "With these tools we can label all the nerves that are innervating a particular organ," Trotman says. "And then we can see the green nerves and the red tumor cells, and how they interact."

Perhaps more importantly, these visualization techniques can help reveal the shortcomings of existing treatments and aid in the development of better ones. For example, the current standard-of-care drugs for prostate cancer—so-called chemical castration medications that stop sex hormone production—makes tumors shrink, but only for a while. "It's a temporary regression, after which the relapse is guaranteed," Trotman says—and medics don't know why. The glowing mice can help shed some light on that. "We want to know what happens to the peripheral nerves that are near the tumor," Trotman says. How does the shrinking tumor rebound? Does it stimulate nerve growth? Is it able to get more nutrients as a result? "Those are the questions we'd like to find answers for."

This work may ultimately help answer other puzzling questions about cancer causes. "For example, prostate cancer is much more prevalent in tall men," Massimo shares—likely because it has something to do with the growth hormones that come from the brain. Does the tumor somehow hijack the growth hormones for its own benefit? Are the nerves involved? If so, can scientists devise drugs that interfere with that process? Perhaps some of these questions can be answered, too.

When it comes to severity and prognosis, prostate cancer risks can be deceptive. Compared to many other aggressive malignancies like brain or pancreatic tumors, prostate cancer usually doesn't spread or kill quickly—many men live 10 years and even longer after their diagnosis. But because it is so common—about 12.5 percent of men get it, according to the National Institutes of Health—overall, it takes a lot of lives. In 2019, 224,733 cases were reported, and 31,636 men succumbed to it. "The problem is that it's so widespread," Trotman says. "Since only 5 to 10 percent of people who have it will develop metastatic prostate cancer, an average patient has a 90 percent chance of being fine. But because so many men develop it, it is still the second cause of cancer death in men, after lung cancer. So if we can prevent or reduce the occurrences of the metastatic disease, we really can save a lot of people."

LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.

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New Veggies for a Warming Planet

We need a diversity of crops to adapt to Earth's changing climate

BY VIVIANE CALLIER

WHEN YOU BITE into an ear of fresh corn, you are eating something profoundly unnatural. A modern ear is a big, flavorful thing packed with 18 rows of plump kernels. Its sad-looking wild ancestor had just six to eight rows of kernels, looking more like something you'd weed out of your lawn than something you'd put on the grill. The juicy version we eat today is the result of thousands of years of breeding and selection. The same is true for most every modern crop: They have been genetically modified over and over to feed an ever-growing, urbanized population.

Now we need to remake our crops yet again. The old strategies of improving size and yield are no longer enough. A couple centuries of human greenhouse emissions have caught up with us. With the world likely to get at least 2 degrees Celsius warmer, on average, by the middle of the century, and with extreme storms, rains, and drought already happening more frequently, growing conditions are changing faster than farmers and their crops can adapt. Zachary Lippman, a professor of genetics at Cold Spring Harbor Laboratory, likens the situation to an arms race—only this time around we're competing against ourselves.

Agribusinesses like Cargill and Archer Daniels Midland are aware of the battle and mostly betting that improved crop breeding, using the latest techniques of genetic engineering, will save the day. Lippman doubts that will be enough. "Winning the climate-change arms race is going to be extremely challenging," he says. Continuing to fine-tune the traits of existing crops, making them even more specialized, could only make the challenges greater. We need to address one of the fundamental weaknesses of modern agriculture: an extreme reliance on just a few strains of just a few crops, notably corn, wheat, rice, and soy.

Solving the food-and-climate crisis will require going back to basics, finding ways to make our mix of crops broader rather than even narrower. "We need to think about how domestication worked in general, thousands of years ago," Lippman says. Back then, early forms of modern crops could be taken out of their

In a warming world, we need to remake our crops. The old strategies of improving size and yield are no longer enough.

original geographical range and grown in new places because farmers and breeders selected mutations that allowed those adaptations to occur. We can do that again, adding overlooked crops to the mainstream food supply and working to broaden the agricultural gene pool after centuries of going the other way. That adjustment will help ensure that farmers will have crops suitable for the extreme growing conditions they are likely to encounter in the coming decades.

"We haven't done a very good job of maximizing diversity," Lippman says. "And diversity is what you need to win the battle of climate change."

TODAY, AGRICULTURAL ALTERNATIVES already exist in the form of orphan crops: ones that are cultivated on a small scale in some parts of the world, but that have not benefited from breeding and research to the same extent that major crops have. Some of them are already suited to relatively hot or dry conditions. Because they have not gone through the same extensive breeding as corn, soy, and wheat, the orphans have more untapped potential.

The first hurdle to embracing orphan crops is identifying the most promising ones and drawing attention to them. Quinoa is a prime example of an orphan crop that languished in obscurity. It was consumed for thousands of years in the northern Andean region of South America, but was little known beyond there. Starting in the 1980s it began to attract attention as a healthy traditional grain, and benefited from extensive research and marketing. It is now becoming mainstream; global production more than tripled, to 230,000 metric tons, between 2009 and 2019.

Many other orphan crops could become increasingly important as climate change destabilizes the existing system of agriculture. Lippman's research group at Cold Spring Harbor Laboratory has been investigating orphans in the *Solanaceae* family, a diverse group that includes tomatoes, potatoes, eggplants, and peppers. At least 25 orphan crops exist in this family, and there are many other uncultivated wild relatives that have crop potential. Lippman is particularly interested in the domesticated African eggplant, grown for its fruit, and a wild relative, *Solanum anguivi*, whose leaves are eaten. Only local communities eat these species, because there hasn't been much interest in developing them into mainstream food crops. "There are dozens of *Solanaceae* plants that have more widespread agricultural potential than we currently realize," Lippman says.

Working with plant biologist Yuval Eshed of the Weizmann Institute of Science in Israel, Lippman has studied domestication genes in tomato plants; they and other researchers have found that domestication produced beneficial effects by altering the same genes across different plant lineages. The set of genes associated with the domestication of many crops direct the production of two key hormones, florigen and antflorigen. These hormones control the timing of flowering and how many flowers are made on each plant, as well as the growth and branching of plant stems. The recent discoveries suggest that focusing on this handful of genes could accelerate the improvement of orphan crops, or even enable the de novo domestication of wild plants with crop potential. Eshed and Lippman are enthusiastic about orphan legumes, such as the drought-resistant chickpea, that have great potential for wider cultivation. Teff, a hardy, protein-rich cereal grown in Africa, is another orphan crop whose production could be vastly expanded.

Lippman suggests thinking as if we were creating modern agriculture all over again from scratch: "We can focus our attention on the genes and families of genes that have been shown over history to have been the most important ones for driving the trait changes that give us the type of agriculture that we have today," he says. "Of course, there are so many more changes in other genes that were also important and also need to also be considered, but certain gene families stand out."

YARUNIC STUDIO / SHUTTERSTOCK

BEYOND QUINOA
Quinoa was consumed for thousands of years in South America, but was little known beyond there. Its recent success with consumers as a healthy traditional grain could foretell the widespread adoption of other regional or orphan crops.

The gene editing tool is a means for us to walk side by side with what nature has already given us, and aid it along.

Florigen-regulating genes have been repeatedly modified by breeders in crops as diverse as tomatoes, soybeans, potatoes, beans, strawberries, barley, sugar beet, rice, and wheat. (Through most of history, farmers were doing this type of genetic modification based on appearance alone, with no awareness of what was going on at the molecular level.) Controlling flower production is critical because flowers become fruit; the timing of flowering determines the length of the growing season, and the harvest. Biologist Akiva Shalit-Kaneh of the Technion–Israel Institute of Technology, working with Eshed and others, finds that florigen and antiflorigen also influence the growth pattern of stems. Tubes in the stems that pump water and nutrients throughout the plant (vasculature) grow and mature in parallel with the process of flowering; this co-regulated growth by florigen and antiflorigen shows that their associated genes can improve crops in other ways.

Instead of waiting patiently for spontaneous beneficial mutations to arise, as plant breeders did for thousands of years, the idea is to speed things up by using CRISPR gene editing to create variations at the locations in the genome that code for florigen and antiflorigen—essentially inducing diversity on demand. Increased genetic diversity at these targeted regions of the genome would enable a wider range of adaptations to a changing climate. Plant breeders could use that broadened genetic palette, in combination with already existing genetic variations, to select plant varieties best suited to particular growing conditions, such as greater heat, more frequent drought, or high salinity.

A group led by Lippman and postdoctoral fellow Choon-Tak Kwon has done exactly that with tomato plants. Working with colleagues in Korea, Israel, and elsewhere in the United States, the researchers have gene-edited tomatoes to flower more quickly and to grow shorter stems, leading to a compact plant suitable for urban agriculture—another promising response to the limits of current agriculture. Growing and distributing food within densely populated areas avoids the high energy and transportation costs of industrial farming, and it can also increase food security in communities that don't have easy access to fresh produce. In another study, Lippman's group engineered 30 gene variants (alleles) in a tomato to modulate the size and weight of the fruits. It was like a dial that could be turned up or down to produce just the fruit-size desired.

In principle, these same genetic tuning techniques can now be applied to other members of the *Solanaceae* family, transforming less-familiar plants into useful new crops. Although the CRISPR approach is cutting-edge, the genetic mechanisms it activates are the same ones that plant farmers have been manipulating for thousands of years—the same mechanisms that the plants themselves have evolved over tens of millions of years of natural selection. "We need to bow down in respect and nod to nature, and how breeders work with what nature provided," Lippman says. "The gene editing tool is a means for us to walk side by side with what nature has already given us, and aid it along."

IDENTIFYING AND IMPROVING orphan crops is just one part of the effort to change the direction of global agriculture. Agribusiness is a $1 trillion industry in the U.S. alone, and corporate farms have little incentive to do labor-intensive fine-tuning of new varieties while their profits depend almost entirely on just a few crops. In fact, American fields are less diverse today than they've ever been, as companies focus on growing corn and soybean; together, those two crops alone account for 180 million acres of planting in this country, according to the U.S. Department of Agriculture.[1] Turning away from mainstream crops to develop obscure new varieties "is not something that private companies will jump on if there's no financial incentive there," Lippman says.

Aubrey Streit Krug, director of ecosphere studies at the Land Institute in Kansas, sees a connection between climate adaptation, economics, and culture, and real change will have to involve all of those factors. "Agronomically, we have to know how to farm these plants, how to grow them well for high yield, how we relate to them and manage them," she says. Farmers will have to learn how to grow and manage the new crops and consumers will have to want to eat them. The produce shelves in the supermarket today look quite a bit different than they did a few decades ago, with quinoa and kale sitting next to white rice and iceberg lettuce, so we know that such transformations are possible.

Krug's colleague Lee DeHaan, lead scientist of the Kernza (wheatgrass) domestication program at the Land Institute, expands on that point. "Genome editing technology by itself will do nothing. It has to live within the context of all this other stuff—traditional plant breeding and all the other work that needs to be done to develop new crops, including food science and agronomy and societal changes," he says. There's no reason that farmers on all scales, from small plots to huge agribusinesses, can't adopt those changes, as long as they have an incentive to do so.

The incentive that could change the way agribusinesses work, in Lippman's view, is that climate change may starkly expose the costs of not changing. Enhanced, super-specialized versions of today's large-scale, single-crop agriculture could leave farms increasingly vulnerable to a harvest catastrophe. We can't predict how the climate will change locally, to match new varieties of engineered crops to that new climate, and we can't reliably predict how engineered plants will respond to unforeseen growing conditions. A single costly crop failure could instantly alter the economic calculations. If Lippman and the other crop-diversity evangelists are successful, though, farmers will have already shifted direction as insurance against such eventualities, embracing both orphan crops and more robust, less-specialized versions of the current staples.

In a 2019 *Science* review paper, Lippman and Eshed examine the different ideas being proposed to feed humanity on a hotter, more crowded planet.[2] "The two main strategies are improvement and further adaptation of the major crops that already benefit from large-scale infrastructure developed around them, and the diversification of agriculture by developing new crops that would better fit climatic changes and address nutritional needs," the authors write. Genome modifications that focus on the florigen system—the same genetic system that led to many of the crop improvements in the history of agriculture—"may yield the greatest return for crop improvement," they conclude.

As much as today's sweet corn is an improvement over its maize ancestor, the supermarket shelves of tomorrow could be a vast advance beyond what we have today. Meat, a major contributor to climate change, is likely to be less common. Foods made from protein-rich cereals and legumes are likely to be a lot more so. Items that now seem exotic, like teff and African eggplant, may have become utterly mundane. But the biggest change will be largely invisible: Future crops will be better prepared for a changing world.

Using CRISPR and associated gene-editing tools, along with still-untapped natural gene variations, breeders will reverse the centuries-long pattern of increasingly specialized, vulnerable corn, rice, wheat, and soy. The strains that replace them will look superficially similar, but they will recover the long-lost adaptability of their distant ancestors. The (former) orphan crops will share a similar hidden secret. They, too, will be the beneficiaries of CRISPR editing—in this case, used to create larger, tastier, more nutritious variants in just a couple generations, rather than the dozens or hundreds required by traditional breeding. We missed the chance to stop climate change, but Lippman believes we can still win the race to remake agriculture and safeguard the food supply for humankind.

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The Rise of RNA Therapeutics

DNA mutations are hard to fix. Scientists are trying another approach.

BY LINA ZELDOVICH

MOST AMERICAN NEWBORNS will arrive home from the hospital and start hitting their developmental milestones, to their parents' delight. They will hold their heads up by about three months. They will sit up by six. And they will walk around their first birthday. But about 1 in 10,000 will not. They will feel limp in their caregivers' arms, won't lift their heads, and will never learn to sit on their own. When their alarmed parents seek medical help, the babies will be diagnosed with spinal muscular atrophy, or SMA, a neuromuscular disease in which certain motor neurons of the spinal cord progressively deteriorate. The disease is triggered by a genetic malfunction that boils down to the gene called *SMN2* (survival motor neuron 2), which causes bits of vital proteins to assemble incorrectly, resulting in progressive muscle weakness and paralysis.

CROCOTHERY / SHUTTERSTOCK

Until five years ago, this diagnosis wasn't far from a death sentence. SMA was considered the most common genetic cause of infant mortality. Many babies with SMA didn't live to celebrate their second birthdays. Some lived past their toddlerhood, but never grew strong enough to run around or play with other kids, and eventually succumbed to the disease. But in 2016 that dire prognosis changed for the first time in history—thanks to a new FDA-approved therapeutic developed by Adrian Krainer, a biochemist at Cold Spring Harbor Laboratory, in collaboration with Ionis Pharmaceuticals and Biogen.

Called Spinraza, the drug fixed the problem in a unique way. Administered through a spinal tap, Spinraza goes to work just as the *SMN2*'s garbled genetic code is transcribed into defective protein-making instructions—and corrects those instructions at the molecular level. Using more scientific terminology, Spinraza intervenes shortly after DNA is transcribed into RNA, a workhorse molecule responsible for many cellular processes, which in this case acts as a messenger carrying DNA's instructions. "Spinraza is designed to bind to the messenger RNA, which enables the cell to handle it properly, and ultimately corrects the problem," explains Krainer, who won a prestigious Wolf Prize in medicine in 2021, for his work explaining the molecular mechanisms behind this RNA process, which led to this new therapeutic.

In 2020, messenger RNA, or mRNA, made the front pages of every newspaper as Pfizer and Moderna used the molecule to create COVID-19 vaccines. With this new method, never used before to vaccinate humans outside clinical trials, the drug makers employ mRNA to deliver specific instructions to our cells. The instructions tell the cells to generate the spike protein that coronavirus uses to infect us. Once produced inside the body, the spike protein draws the ire of the immune system, which remembers it as a foreign invader and is primed to fight the real coronavirus. After a while, the cells also destroy and remove any trace of the vaccine's mRNA. The mRNA technology appeared novel, but it had been in the works, though out of the limelight, for years.

For decades, DNA has been scientists' primary focus, while RNA was merely viewed as a helper, a passive carrier of genetic instructions, just an intermediary between DNA and proteins. "When it came to RNA, even scientists weren't clear what was so important about it," says Lynne Maquat, who leads the Center for RNA Biology at the University of Rochester and who shared the Wolf Prize with Krainer. "People thought there were only three kinds of RNA, we already know what they do, end of story."

Researchers think RNA has huge untapped therapeutic potential.

But that view has changed. Not only did scientists discover many different types of RNAs, but they also realized that a huge portion of our DNA is devoted to making them. "We now know that at best only 3 percent of our genome codes for proteins," says Joan Steitz, professor of molecular biophysics and biochemistry at Yale University, and another co-recipient of the Wolf Prize, who has been studying RNA since the 1960s. "And the other 97 percent is devoted to making all these different kinds of RNAs. We know what the most abundant and important of them do, but there are thousands of different ones that we still don't have a full understanding of." Building this understanding holds keys to treating many genetic disorders, which may originate in the faulty DNA, but can be corrected by mending the RNA or the processes in which this RNA is involved.

Researchers think that RNA has huge untapped therapeutic potential. Traditionally, when developing new medicines, pharmaceutical companies target malfunctioning proteins that cause disease. But targeting RNA allows the problem to be corrected one step earlier, before the proteins are made, says Justin Kinney, a quantitative biologist at Cold Spring Harbor Laboratory who collaborates with Krainer to understand the inner workings of this molecule. "RNA is a great target for drug development," Kinney says—because it is so versatile. His goal is to build a roadmap to a new generation of RNA-based therapeutics.

Our understanding is so limited scientists sometimes don't know why a drug works.

IT'S NOT OVERLY SURPRISING that the DNA molecule overshadowed its less glamorous cousin for over half a century. After all, DNA's charismatic double-helix string, tightly woven and nestled inside the cell nucleus, holds the code of life. Like a queen bee, the DNA molecule runs its cellular kingdom, dispatching orders for a myriad of cellular functions. Scientists who aimed to unveil the root-cause of genetic disease focused on the DNA.

But no queen can run her kingdom alone. A queen needs her messengers, maids, guards, and attachés. And that's where the RNAs come in. Like their queen's emissaries, RNA molecules carry out the instructions for protein assembly, catalyze reactions, and perform other duties, keeping their cellular dominion in good health.

If you pictured each and every one of your cells as a bustling kingdom, you'd see a gazillion RNAs teeming around at all times. You would see the DNA being transcribed—its genetic instructions copied into messenger RNAs. These mRNAs would pass these instructions onto the ribosomes, the cellular protein, and peptide-making machines, which would assemble them accordingly. To keep the conveyor going, transfer RNAs would deliver amino acids to this protein assembly line. And the specialized ribosomal RNAs would help stitch these amino acids into protein molecules. Meanwhile, more mRNAs are being forged—and just as they are produced, they are also getting spliced and diced for reasons scientists aren't sure about. This is just one of the mysteries on which Kinney's research might shed some light.

The act of transcribing DNA into mRNA begins when an enzyme called RNA polymerase binds to the DNA and starts copying the DNA sequence into an RNA sequence. But what comes out isn't a very usable "draft." For starters, the resulting mRNA is about 10 times longer than it should be, so it must be trimmed—or spliced, a process in which certain parts are kept and others are thrown out. This splicing is done by molecular machines called spliceosomes and involves removing the unnecessary nucleic acid sequences called introns (from "intervening" snippets) and stringing the remaining pieces, called exons, together.

"You can think of the RNA polymerase as a newspaper reporter and the spliceosomes as a very, very stringent editor that cuts 9 out of 10 paragraphs the reporter writes," Kinney explains. "And it's confusing why you would hire such a stringent editor to begin with—can't your reporter just write less? So splicing seems like a very wasteful process. There are still debates about why it even evolved in the first place."

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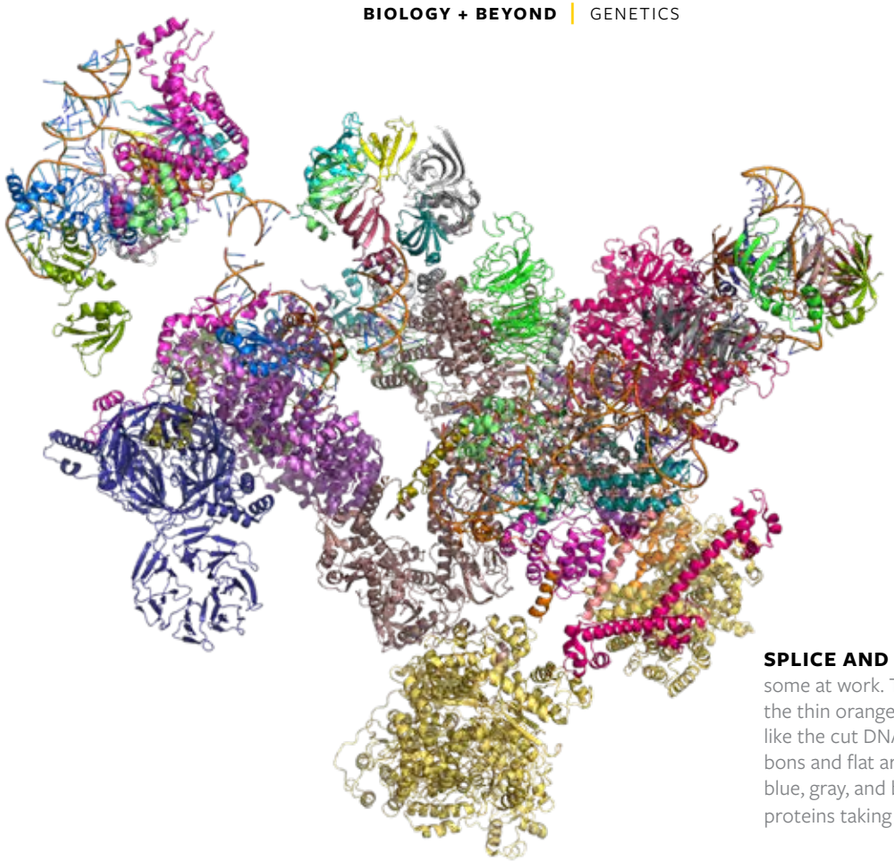


SPLICE AND DICE The spliceosome at work. The RNA in progress is the thin orange string that looks a bit like the cut DNA helix. The curly ribbons and flat arrows—purple, green, blue, gray, and brown—are various proteins taking part in splicing.

COURTESY OF ADRIAN KRAINER / CSHL

The prevailing hypothesis is that it allows for creating a greater variety of proteins, says Steitz, whose research elucidated the splicing mechanism—and human bodies need all of those proteins to function. Among other things, she found that the splicing process itself is governed by yet another RNA player—the tiny RNA-protein particles called snRNPs, or snurps. They find and remove these introns from the mRNA molecules.

In people with spinal muscular atrophy, this process hits a glitch. As introns are removed during splicing, one exon also gets axed from the resulting *SMN2* RNA—exon 7. And without that exon, the proteins assembled with these RNA instructions come out defective, leading to spinal muscular atrophy.

Krainer likens the process to a cookbook with messed-up pages. "Our genome is like a library where thousands of books contain recipes for protein-making, with every chapter spelling out precise instructions, and in the right order," he says. But in between the chapters there are extra pages (the introns) that shouldn't be there. Splicing removes those pages, making reading straightforward. "If splicing is correct, you end up with perfect instructions. But in the case of *SMN2*, there's a defect in Chapter 7, so splicing removes the entire chapter. Now a part of your instructions is missing, and you can't follow the recipe."

And that's where Spinraza comes in, wielding its magic at the splicing level. The therapeutic is essentially a short piece of a DNA-like string, which binds to *SMN2* RNA before that RNA is spliced. As it binds, it blocks various other proteins from messing up the splicing—and that allows exon 7 to be included. The resulting mRNA contains the correct protein-assembling instructions. The first approved drug of its kind, Spinraza is paving the way for other RNA-based therapies—and for good reasons.

RNA-BASED THERAPEUTICS can have a big advantage over the traditional protein-based ones. Currently, drug developers target malfunctioning proteins, aiming to fix their faults. But that's a very intricate and failure-prone process, in which three things must come true, Kinney says. First, the drug must be able to bind to a spot, or a site, on the protein molecule. Second, it must correct the protein's rogue behavior—for example, shut off the protein's active site and disable its ability to cause harm. And lastly, it must not interfere with any other protein in the body, to avoid gumming up other vital functions.

"That's a very difficult problem to solve," Kinney says, because "most proteins don't have a lot of potential binding targets." RNA, on the contrary, is covered with binding sites because it is designed for other molecules to latch onto it. "The whole RNA is a target for drugs," Kinney says. "The only limiting thing here is our understanding of how the RNA is controlled by various regulatory programs within the cell."

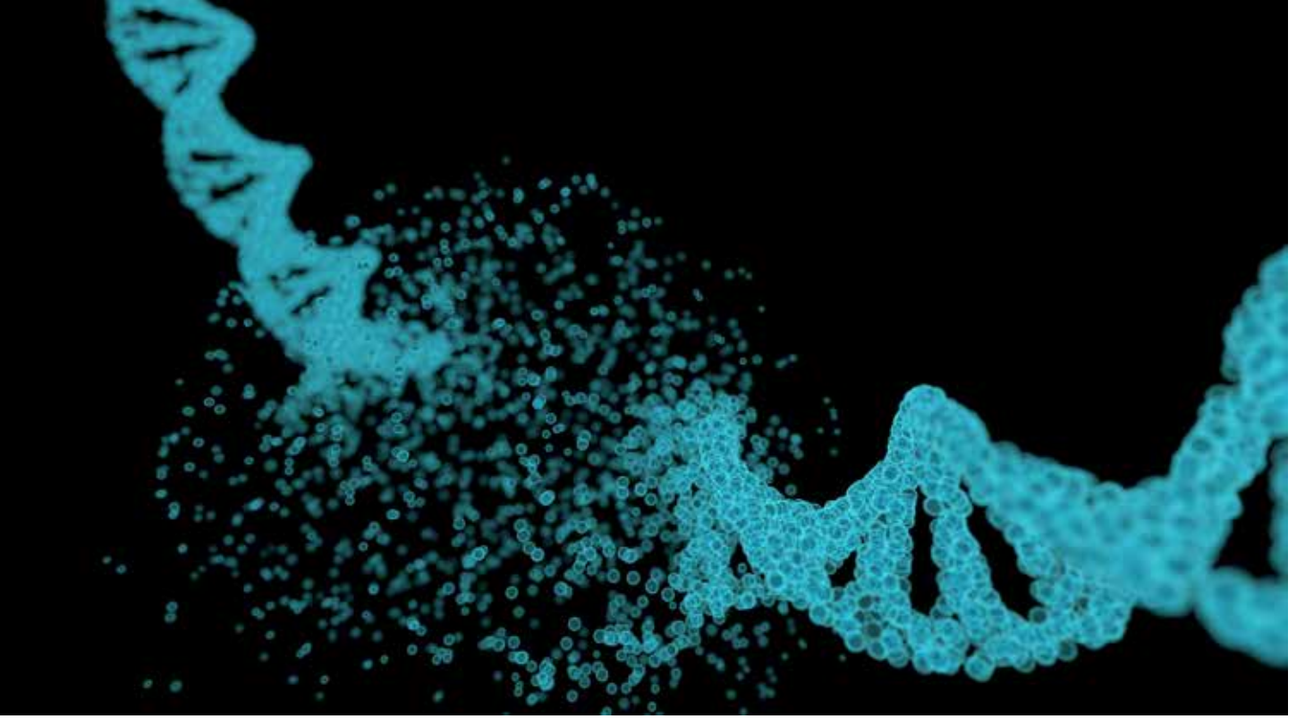
Our understanding is, in fact, so limited that scientists sometimes don't know why a drug works. Kinney cites one example. In 2020, the FDA approved another spinal muscular atrophy drug, Evrysdi. Compared to Spinraza, it's a small molecule, only the size of about one base pair of DNA, and easier to make and administer—it can be taken orally. "It was essentially developed by trial and error," Kinney says. "Scientists took a few hundred thousand random molecules and tested each one in cells to see which ones increase *SMN2* exon 7 splicing. The initial candidate molecule then underwent years of testing and tweaking." Although the final drug, Evrysdi, is safe and effective, scientists are still debating how it functions at the molecular level—in particular, how it singles out *SMN2* exon 7 among the vast number of other exons.

Splicing is one of the most complex processes that occurs in human cells, and the spliceosome is the most complex piece of cellular machinery identified so far. In addition to the snRNPs, the spliceosome includes over 100 different proteins. Incredibly, all of these interlocking molecular parts must assemble anew at each RNA intron. Decades of research unveiled how this complex machine operates once it assembles, but less is known about how the spliceosome recognizes the specific chunks of RNA it must cut out. "Understanding this requires new quantitative approaches," Kinney explains.

Our genome is like a library where thousands of books contain recipes for protein-making.

That's what Kinney is working on. Using high-precision experiments, mathematical modeling, and artificial intelligence, Kinney aims to clarify these mysteries at the level of molecular biophysics—how the spliceosome reads the RNA sequence and makes its cutting decisions, and how drugs like Evrysdi zero in on their specific targets. Splicing contributes to many diseases, including cystic fibrosis and cancer, and even those to which it doesn't contribute, Kinney says, "may be treated by modulating the splicing process." Once scientists elucidate the molecular cogwheels involved in splicing, they can determine where they get off track and correct them. "And that," Kinney says, "will open a lot of opportunities for making new and better drugs."

LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.



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A Universal Cancer Treatment?

A medicine that disrupts the DNA replication of cancer cells may be within reach

BY LINA ZELDOVICH

IMANSHU BRAHMBHATT was staring at the results of a clinical trial that looked too good to be true. A co-founder and CEO of EnGeneIC, a biopharmaceutical company, Brahmbhatt was running a small trial that was testing a fundamentally different approach to fighting cancer. Patients in the group had grim prospects. They had exhausted all other options. With nothing left to lose and not expecting any miracles, they enrolled in the trial. They wanted to give it one more chance. Now their scans showed their tumors had stopped progressing. Even more remarkable was they didn't have the same type of tumors. They had malignancies affecting different organs—lungs, bladders, colons, pancreases—and yet, they uniformly did well.

They sent a Trojan horse into cells and turned cancer's own trickery against it.

"These people were facing death," Brahmbhatt says. "Then we started seeing that they were actually succeeding. You could see in the scan that the tumor has stopped growing. It was a feeling of such extreme internal joy that it's very difficult to describe."

The results may have appeared miraculous, but they were anything but. They stemmed from fundamental research into cell division that forms the basis of the EnGeneIC process. A longtime advisor to the company, Bruce Stillman, professor of biochemistry and president and CEO of Cold Spring Harbor Laboratory, has been studying the process of DNA replication, which plays a key role in cell division and cancer progression.

"Cancer cells multiply out of control," says Stillman, who has devoted his career to studying DNA replication. "When a cell becomes a cancer cell, the very first thing that happens is the cells begin to divide without the normal controls. And the first thing that has to happen before the cell has to divide into two daughter cells is to copy the genome. So, the path that leads to cancer is in part dysregulation of the process that controls DNA replication." The abnormal DNA replication causes the accumulation of mutations in the genome that advances cancer. Interfering with the process of cell division has long been a focus for treating cancer, but because normal cell division is unavoidably affected also, many of these chemotherapies are toxic. EnGeneIC has figured out a way around this problem by combining a novel method of drug delivery with a way to stop DNA replication.

Stillman was a graduate student when he understood that cell division, and DNA replication in particular, were key targets for treating cancer. That insight inspired him to switch careers from medicine to medical research. At the time, the science of DNA was a burgeoning field and there was a lot to discover; Stillman was a pioneer. He uncovered many of the mysteries of the genome replication process and what sets the copy machinery in motion. He has spent 40 years putting together the pieces of the molecular puzzle. "I wanted to understand how this process really works," he says. And he did.

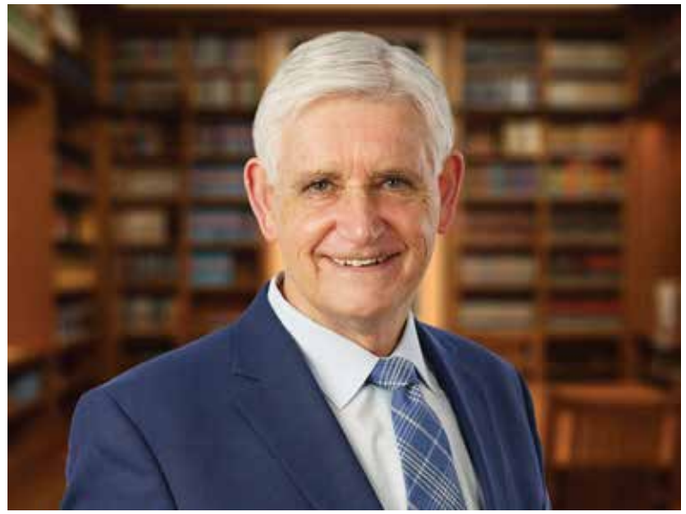
DEEP INSIDE the tens of trillions of cells that comprise your body, the DNA replication machinery is constantly speeding along in many tissues. In the bone

marrow alone, 500 million red and white blood cells are produced every minute. There's about two meters of DNA in each cell, neatly woven inside the nucleus. To keep the blood cell supply steady, about a billion meters of DNA must be copied every minute. "You could wrap that around the Earth along the equator about 25 times," Stillman says. It is inevitable that over the course of a person's lifetime, this process will make mistakes—some harmless, but others leading to malignant mutations. So, understanding the cogs of this complex machinery may hold the key to combating many cancers.

Stillman and his team discovered that the replication process starts with a set of six specific proteins called Origin Recognition Complex, or ORC. The proteins bind to the DNA at specific locations and recruit more proteins to help, forming what's called the pre-replicative complex. This pre-replicative complex "gives permission" to start DNA replication and many proteins begin copying the genetic material from their respective starting points. Once the job is finished, the pre-replicative complex is destroyed. Once the cell is ready to divide again, the complex is formed anew.

Stillman's group first discovered the ORC in yeast, experimenting with a strain called *Saccharomyces cerevisiae*, normally used to make bread, wine, and beer. (Notably, in 2020, he won a Heineken Prize for Biochemistry and Biophysics for his work, although it didn't have anything to do with brewing—the prizes are awarded to the leading figures in the above disciplines as well as in art, medicine, history, and environmental and cognitive sciences.) As Stillman's team kept studying the ORC phenomenon, they realized this ancient process works very similarly in humans. "It's remarkable to think that yeast and humans share the same process of DNA replication," Stillman says. "We started off with yeast cells and we were able to walk through evolution and eventually get to the human genes."

Another key player in DNA replication is an enzyme called DNA polymerase. DNA polymerases synthesize the new DNA by adding nucleotides—the gene building blocks—one by one to the growing DNA chain. "There are about 15 polymerases involved in this procedure," Brahmbhatt says. "Each of these polymerases does a different job inside that DNA replication process." If some of them stop working, the cell won't be able to divide.



DNA DETECTIVE Bruce Stillman has devoted his career to studying DNA replication. He believes that cell division, and DNA replication in particular, are key targets for treating cancer.

Knowing the key points in DNA replication has allowed scientists to ponder ways to jam the copying gears inside the rogue cells. At the biochemical level, gear-jamming isn't an insurmountable task—today scientists can forge a small molecule that can bind to some of the players and "disable" them. The real challenge was to deliver these molecules inside the cancer cells and no others. If these molecules ended up inside healthy cells, they would halt the normal replication processes, killing the cells—and the person with them.

SCIENTISTS KNOW cancers have an uncanny ability to travel through the body by slipping in and out of blood vessels to hitch a ride through the bloodstream. They do it by loosening the arterial walls and squeezing through. "Normally, our blood vessel walls are all sealed pipes," Brahmbhatt says. "But around wherever the cancer is growing, the blood vessels are known to be very defective. They have got a lot of holes in them."

PHOTO COURTESY OF CSHL

It was a feeling of such extreme joy that it's difficult to describe.

Brahmbhatt and his collaborator Jennifer MacDiarmid devised a clever ploy. They would send a Trojan horse into malignant cells and turn cancer's own trickery against it.

The Trojan horse, in this case, is a product of a harmless bacteria that's been genetically engineered to have specific qualities. When this genetically engineered bacteria divides, it yields a tiny non-living cell of 400 nanometers in diameter—the right size to slip through the damaged vessels and mingle with the tumors. To the nano-cell, Brahmbhatt and MacDiarmid affixed a "molecular hook" that the tumor grasps and swallows. The nano-cell is also packaged with "interfering" RNA molecules—distant relatives of the RNAs used to create the first coronavirus vaccines. Known as siRNAs, the molecules interfere with the expression of specific genes. They can target some of the DNA replication enzymes and inhibit their function. Brahmbhatt says Stillman provided invaluable advice about which polymerases to target to kill malignant tumors.

Brahmbhatt and MacDiarmid tested the nano-cell assassins in mice and then in a small human trial. "There was a big fear at first," Brahmbhatt says. "Everybody was frightened because these molecules have never been sent specifically into a human, and everybody knew that if this thing gets into the wrong cells—meaning into the normal cells—it could be fatal." That's why the study's first cohort included only people who had exhausted all other options.

When he looked at the first set of scans, Brahmbhatt recalls, "what we saw was something fantastic. With these molecules, which are inhibitors of DNA replication, we could extend their lives." More surprising was the fact that—unlike systemic chemotherapies—there were no toxic side effects. Currently Brahmbhatt's company, EnGeneIC, is preparing for the next phase of clinical trials.

Should the siRNA method prove its worth in larger trials, the public health impact will be much greater than just putting another anti-cancer compound on the market. Stunting DNA replication has the potential to become cancer's universal treatment. The research Stillman did, as well as others who chose to study the fundamental biology of DNA replication, is now not only bearing fruit, but—combined with the right delivery system—has the potential to change the cancer treatment paradigm. And that's the beauty and importance of the fundamental research, without which modern clinical applications would be impossible. "Today, those discoveries have become critical," Brahmbhatt says. "If this works, we won't just cure one type of cancer. We would cure cancer across the board."

LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.

ANEK SANGKAMANEE / SHUTTERSTOCK

The Race to Protect Sweet Corn

Breeding a variety that can withstand disease and taste better, too

BY LELA NARGI

WE MAY NOT ALWAYS call it sweet corn, but we enjoy this American staple nearly daily. It's sweet corn that we grill on the Fourth of July, charring the golden ears to get a smoky tang. It's sweet corn that we buy at summer farmers' markets to boil and smother in butter and salt. And it's sweet corn that pops into the beloved crunchy comfort food we crave on movie nights. It's the type of crop we'd like to stay healthy and plentiful. But climate change—and longer periods of wet cool weather—can turn sweet corn to rust.

Climate change and long periods of wet cool weather are turning our favorite movie-night munchie to rust.

For sweet corn growers, it's a familiar annoyance: oval-shaped, cinnamon-brown pustules, known as common rust, that appear on corn leaves and bode ill for that season's haul. Caused by the fungus *Puccinia sorghi*, it is a prevalent disease for our staple of summertime picnics and backyard barbecues. In the United States and other places sweet corn is grown, it can devastate crop yields, and while we haven't yet hit a sweet corn shortage, the disease can cost farmers a pretty penny.

Other corn varieties, which comprise about 99 percent of corn production in the U.S, aren't impervious to disease either. They can be plagued by a list of other ills that includes southern rust, tar spot, sugarcane mosaic virus, northern corn leaf blight, and maize lethal necrosis disease. But unlike many of these other varieties grown to feed cows or make the syrup used in processed food, sweet corn, in general, is not genetically modified. Plant biologists have discovered that it's more vulnerable to common rust than its other corn cousins. So as climate change shifts rainfall patterns,[1] which leads to damper-than-usual conditions or flooding, and creates unseasonably fluctuating temperatures, common rust is fixing to be a bigger problem for sweet corn in the U.S.[2]

Currently, conventional growers of sweet corn can use chemical fungicides, such as azoxystrobin, to fight common rust. But these chemicals can be highly toxic to animals that live in water and have the potential to harm microbial life in soil,[3] making them less-than-ideal "fixes," and the United States Department of Agriculture (USDA) prohibits organic sweet corn growers from using many of them. Pinpointing the genes that increase resistance to common rust can help build healthier crops. So sweet corn breeders are looking for genes they can add to their breeding lines to build in resistance to common rust.

Luckily, these breeders have an ally in Doreen Ware, a computational and molecular biologist at the USDA Agricultural Research Service, and an adjunct professor at Cold Spring Harbor Laboratory. For decades, she's been using bioinformatics and molecular tools to map the corn genome—and, critically, making this information open source, so even public breeders with limited budgets at land grant universities can access it for free. Ware explains that in the last 20 or 25 years, scientists have been able to achieve a deeper level of granularity in their understanding of genomic structure—for corn and many other crops—due to sequencing innovations in the medical and health arenas. "We've been able to leverage everything that's been done for human AI and do it on the cheap for plants," Ware says.

Ware's research makes life considerably easier for agricultural scientists like William Tracy, at the University of Wisconsin-Madison's agronomy department, who develops seeds for seed companies that eventually make their way into the hands of farmers and gardeners. Historically, Tracy would have spent seven or eight growing cycles to come up with a corn hybrid that was extra-sweet, or resistant to any one of a variety of diseases that plague the crop. He'd select two parents, mate them, pick the progeny that showed the most promising traits, mate and grow them out again, and again, and again. "Old-fashioned plant breeding is iterative," he says. "We make a gain, then cross the things we made with that gain to make more gain." That can take a long time.

Thanks to Ware, the present and future of breeding is quicker and a lot more precise. Her sequencing work has given Tracy access to genetic information that provides significant shortcuts in coming up with new hybrids. By being able to pinpoint valuable genetic traits and where they lie, he and his team can conduct

SWEET AND STRONG
Doreen Ware is examining young corn plants in hopes to make our favorite crop resistant to diseases that might arrive with climate change.

COURTESY OF DOREEN WARE

fewer crosses and trial grow-outs in the field, reducing the number of growing cycles from eight to five or even three in some cases, and cut down on the expense and time it takes to get improved seeds into the hands of farmers. Using those tools, "I'm confident we can develop material that is going to be helpful in dealing with climate change," Tracy says.

Ware co-authored a paper published in the journal *Science* in 2021 outlining the results of her most recent work of sequencing 26 corn lines used in breeding, chosen because they represented a large swath of corn's genetic diversity.[4] Sequencing yielded over 103,000 different pan-genes, one-third of which were found across all 26 lines, and were dubbed as a "core set." (Previously only 63,000 maize pan-genes had been known, so Ware's work filled a lot of knowledge gaps.)

And among the remaining two-thirds of pan-genes—those that do not repeat across all 26 lines—lie potential answers to making corn varieties more resistant to disease.

Breeders don't yet know what answers these particular pan-genes will offer, but they are excited about future discoveries. According to Tracy, molecular geneticists still have to figure out where any possibly important genes are located. Once they do, breeders can figure out which pan-genes, in which lines, they can cross with existing lines in order to get the desired traits.

BIOLOGY + BEYOND | GENETICS

The genetic wisdom of molecular biologist Doreen Ware is helping farmers defeat corn disease without chemicals.

There's one exception, though, Tracy says. Scientists already figured out two corn lines that contain the gene *rp1*, which codes for the resistance to common rust. He's looking to get a hold of one of those lines, called M37W, in order to cross it to some of his existing lines. Using this in tandem with other computational tools Ware has developed, Tracy says he can have one of his grad students "sit at a computer terminal and look at the genomic data and say, 'Ah, these six genes are important for [disease] resistance.' " Previously, Tracy would not have had much access to information about these structural variants, but thanks to the sequencing outlined in the *Science* paper, that's changing.

This newly elucidated genetic wisdom has more to offer. As Tracy gets ready to send away for the M37W seeds to cross-breed with other sweet corn lines, he awaits more genetic information that will allow him to tackle other diseases afflicting other corn varieties—from maize lethal necrosis disease to sugarcane mosaic virus to northern corn leaf blight, which he says, is becoming vastly more common with climate change.

But there's another, seemingly frivolous, but tasty, benefit to the work Ware has made available to him over the years: It's provided him with the genotype that codes for sweetness in sweet corn. "I used to have to bite 300 ears of corn hybrids in a day to evaluate them for flavor, which gets old," Tracy says. "With molecular and genomic predicting tools, I can eliminate many of the worst ones without tasting them, which means I can be faster, more precise, and not so cranky at the end of the day." And at the end, the sweet corn will taste even sweeter.

LELA NARGI is a veteran journalist covering science, sustainability, and food policy and agriculture, for outlets such as *The Guardian*, *Washington Post*, *Hakai*, *The Counter*, *JSTOR Daily*, and *Ensia*. Find her at lelanargi.com.

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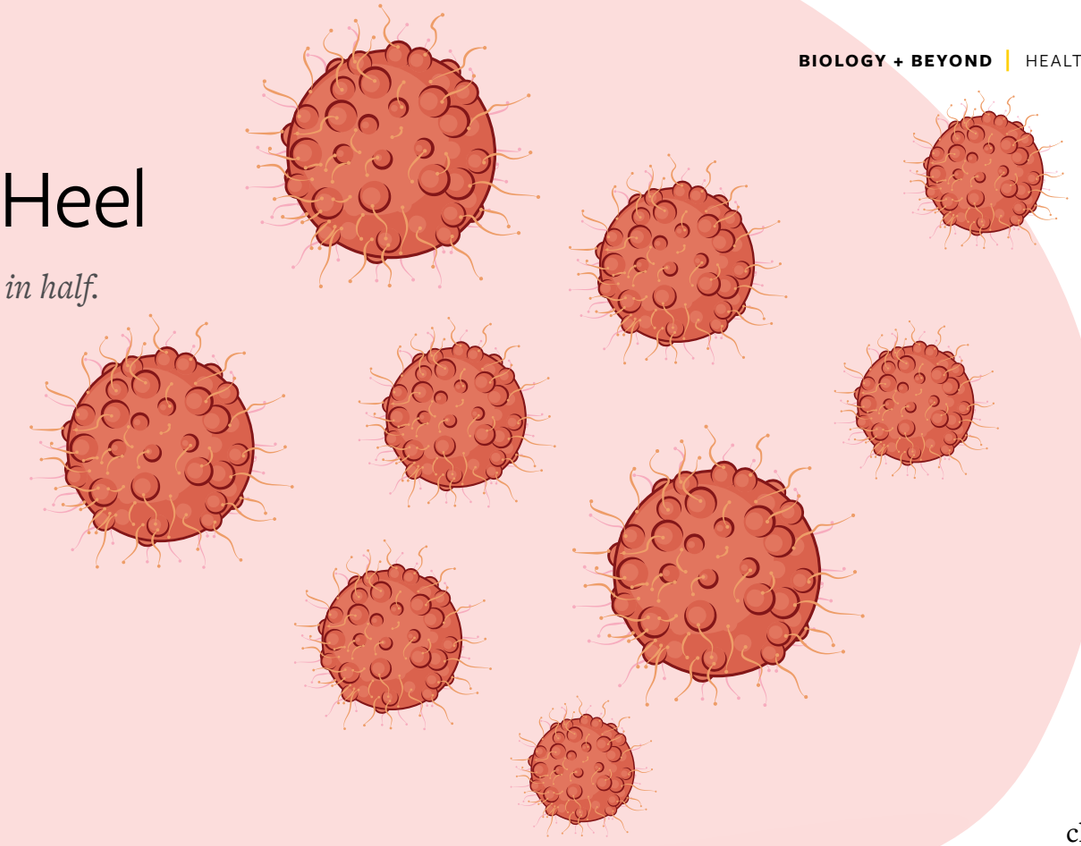
Targeting Cancer's Achilles Heel

President Biden's Cancer Moonshot aims to cut annual deaths in half. Scientists have the goal in their sights.

BY LINA ZELDOVICH

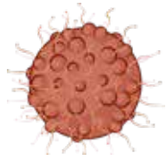
LE PANDA / SHUTTERSTOCK AND HUNKY / SHUTTERSTOCK

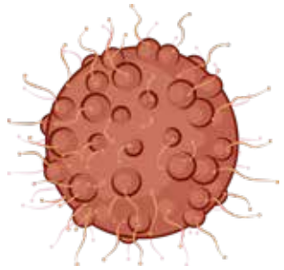
BIOLOGY + BEYOND | HEALTH



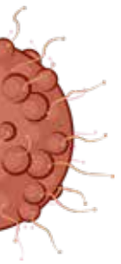
MATTHEW WEISS dreams of the day when his oncology practice will operate very differently. A surgeon at the Northwell Health System in New York who treats pancreatic cancer—one of the deadliest malignancies known—he doesn't have a lot of choices when it comes to saving his patients. Some people with pancreatic tumors die within a few weeks and others fight longer, but only 11 percent of them are still alive five years later.[1] Current treatment options are limited. There are only two treatment paradigms, one based on a cocktail of two chemotherapy drugs and another one based on three, but doctors never know which one will work. "We may as well flip a coin when we decide which regimen to use," says Weiss. "We have no way to predict who's going to respond to what chemotherapy."

Doctors can of course switch from one regimen to another if the initial performs poorly. But it takes a few months to determine whether tumors are shrinking or not, and patients don't have that time. Moreover, the initial chemo may sicken some to the point that they are too weak to try the second approach. To make things worse, the incidences of pancreatic cancer, once considered rare, have increased in the past 20 years, now reaching over 60,000 cases annually in America alone.[2] "About 1 in 50 to 60 people get it, and most of them don't survive it," says David Tuveson, cancer biologist at the Cold Spring Harbor Laboratory and director of CSHL's Cancer Center. "It used to be rare, but it's not anymore. It is now the third most common cancer, behind lung and colon, and predictions show that it's going to take the number two spot soon."





To battle cancer better, treatment should be tailored to patients' individual needs.



Even more concerning is the fact that pancreatic cancer now strikes early, afflicting a greater number of young people, particularly women under 35.[2] "I saw a patient yesterday morning who is 41 years old, with pancreatic cancer and three young children at home," Weiss shares. With an average prognosis of about five years to live, it's a family tragedy. "Five years is not enough, so to me pancreatic cancer is an emergency," Weiss says. "We have to do things radically different." Treatment must take guessing out of the process.

Weiss dreams of having a way to test the regimens before placing people on them. For the past few years, he has been working on making this a reality in collaboration with Tuveson. Their method involves trying the chemo cocktails on pancreatic tumors *outside* the patient who developed them. In scientific terms such outside tumors are called organoids. They're grown from the small chunks of cancer cells biopsied from patients as part of the diagnosis. Because they retain the characteristics and mutations of the original tumor, their behavior when grown in a lab may predict their behavior inside their human host, including the reaction to the two chemo cocktails oncologists use to treat pancreatic cancer.

If the method works, it will improve patients' current survival chances. But the idea has far-reaching potential. It may revolutionize how doctors treat cancers in the 21st century.

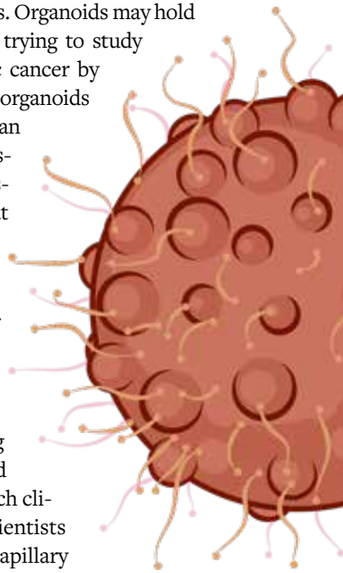
CANCERS ARE TRICKY. Patients respond differently to the same treatments. One beats cancer and the other dies quickly. To battle cancer better, the treatment should be tailored to patients' individual needs—the so-called personalized medicine concept that has taken a center stage in cancer care. Organoids, combined with the genomic data from patients, might provide that much-needed personalized care. These little living tumors can also inform scientists how cancer develops, what changes it causes in the body in early and late stages, and how it plays out in people of different ethnic origins. That can shift not only the treatment approaches, but the entire cancer care paradigm.

In February 2022, President Joe Biden rekindled his Cancer Moonshot effort, which aims to end cancer as we know it. The initiative set several goals, including reducing death rates by at least a half in the next 25 years while improving the quality of life for those battling the disease. Currently, cancer kills about 600,000 Americans a year, so cutting it in half is a multi-pronged challenge. "The White House announcement makes you think, 'What do we need to do to achieve that?'" Tuveson says. He sees the three pillars of success as better prevention, better detection, and better treatment.

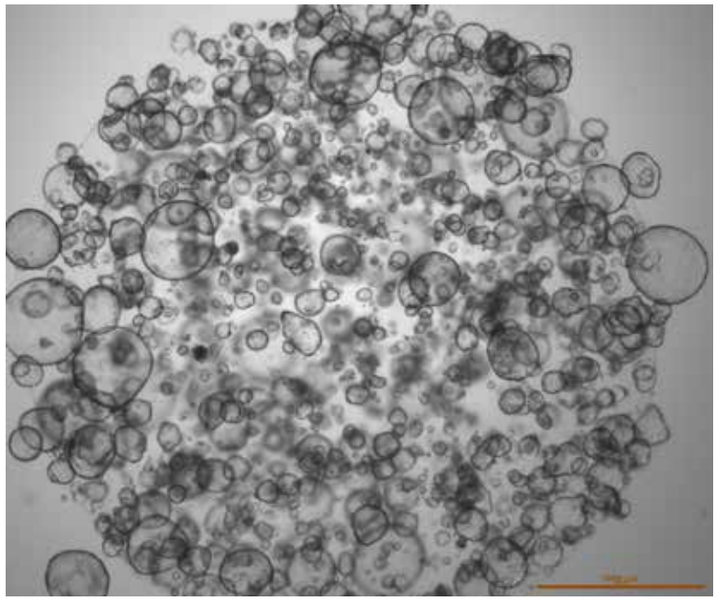
Better prevention begins with understanding cancer triggers, which in case of the pancreas can begin in the complex interplay of the digestive organs. Your stomach digests food and absorbs the nutrients needed to sustain the body, but it can't do all this hard work alone. It does it in concert with the other neighboring organs, including the pancreas and the liver. The banana-shaped pancreas produces the hormones insulin and glucagon, which regulate levels of blood sugar. It also makes the digestive enzymes amylase, protease, and lipase, which respectively break down starches, proteins, and fat. Once made, these enzymes pour through the pancreatic ducts and into the upper part of the small intestine, called the duodenum, where they help digest food. The liver aids with fat breakdown by producing bile, which accumulates in the gallbladder, and then trickles into the stomach via bile ducts. "Bile is like soap, a green, greasy material that dissolves fat," explains Tuveson.

This normally well-oiled machinery can get thrown off track by inflammatory processes. One of them is pancreatitis—an inflammation of pancreatic tissues, which can be caused by solidified bile, more commonly called gallbladder stones. "Sometimes when a stone passes from the gallbladder into the intestines, it may go the wrong way and get stuck in the pancreatic ducts, making the pancreas swell like a balloon," Tuveson explains. "Now the enzymes can't get out, so they start digesting the pancreas itself. And we don't know why, but almost everybody who develops pancreatic cancer has had either symptomatic or silent pancreatitis before it happens." Other things like alcohol overuse, smoking, obesity, and certain medicines can trigger pancreatitis also, which can increase cancer risk. "Many more patients develop pancreatitis than pancreatic cancer, so if we can prevent pancreatitis, we will probably also decrease the number of people who develop pancreatic cancer," Tuveson says.

Organoids might help decipher the differences in cancer behavior between various ethnic groups, says Jeff Boyd, who directs the Northwell Health Cancer Institute's Center for Genomic Medicine, while also holding a professorship at Cold Spring Harbor Laboratory. For example, pancreatic cancer outcomes are worse for African American and Latino people compared to those of European descent. It could be because of diets, pollution, or lifestyle—or due to some biological differences in the pancreatic cells. Organoids may hold the answers. "We've been trying to study the disparity of pancreatic cancer by culturing pancreas cancer organoids from individuals of European ancestry, African ancestry, and Hispanic ancestry," Tuveson says. "So that we could investigate if there's any fundamental difference in the biology of tumors from patients of different racial and ethnic backgrounds."



Better detection requires better imaging procedures or specialized blood tests, neither of which clinicians have right now. Scientists know that an intraductal papillary mucinous neoplasm (IMPN)—a tiny growth arising from the duct lining—can be a precursor for pancreatic cancer, but spotting them early is difficult. Hidden behind the intestines, the pancreas is very hard to image, even with modern MRI and CT machinery. It doesn't help that the pancreatic tumors are usually microscopically small, compared to, for example, an intestinal polyp that gastroenterologists can identify with the naked eye. "An early pancreas tumor is the size of a grain of rice or even smaller," Tuveson says, so they are easy to miss on a digital image. Inserting a camera scope into the pancreas through its very narrow ducts is an impossible feat because it would stop the flow of enzymes, which



WATCH ITS BEHAVIOR
This human tumor "organoid" line was derived from a biopsy of a liver metastasis of a pancreatic cancer. Because an organoid retains the characteristics and mutations of the original tumor, its behavior when grown in a lab is opening a new window for scientists on how a tumor functions in a body, and how to better treat cancer.

COURTESY OF CAITLIN TSANG

would immediately start digesting the pancreas' own tissues. A smarter way would be to develop a blood test that checks for certain compounds that a precancerous pancreas may produce, but so far scientists don't know of any.

That's another problem Tuveson is trying to solve with organoids. His team grows IPMN organoids of these "early cancers" and implants them into the pancreatic ducts of mice, trying to see if this would spark any changes in the animals' blood composition. Tuveson is also using mice implanted with IPMN cells to test new ways to image tiny tumors. "We're growing early human cancers inside the duct of a mouse pancreas, so that we can find new ways to look for evidence in blood or develop new ways to image the cancer," he says. "We use organoids in these futuristic kinds of approaches as a way to develop early detection methods."

Better treatment is where organoids really get their chance to shine. And to some extent they are shining already, as a part of a bigger multi-institute and international effort—a clinical trial called PASS-01, which stands for Pancreatic Adenocarcinoma Signature Stratification for Treatment. When Weiss takes a biopsy, he sends the little cancerous bits to Tuveson's laboratory where they are grown into organoids in petri dishes. Then, Tuveson's lab subjects the patient's organoids to both chemo regimens, noting which one kills cancer more efficiently. Meanwhile, Weiss's colleagues treat the patient on the chemo regimens. After a while, they compare results and see whether the patients' tumors and the organoids reacted to treatment the same way. The team hopes to use this clinical trial to show the first scientific proof that organoids can indeed predict how tumors will respond inside the human body.

At this early clinical stage, the two collaborators aren't using the organoids to decide which treatment to prescribe—they are only trying to establish the correlation. But so far, the results are encouraging. "We have a series of anecdotes that are promising," Tuveson says. "And when you line up a bunch of anecdotes, you suddenly have a trend." When the trend continues, and there are enough cases to undergo rigorous statistical

Organoids give clinicians the flexibility to experiment.

analysis, there's a case for the next level of a clinical trial: Using organoids to predict the best therapy and then treating the patient with it.

Another gift that organoids would give clinicians is the flexibility to experiment. With cancers in a dish, doctors can experiment all they want. They can alter drug ratios, such as decreasing the dose of a more toxic drug to see if the cocktail works just the same. They can swap one drug for another, including those not normally used in the current regimens, and see if it works better. And they can add another medication that's not typically used for pancreatic cancer at all—and see if that works any miracles.

Tuveson likens the approach to shooting arrows at the enemy and finally finding its most vulnerable spot. "What we are doing is essentially looking for the cancer's Achilles heel," he says. And with the ability to test all kinds of approaches on organoids grown in a dish, there's no limit to the number of arrows scientists can shoot. Oncologists will be able to do all the trial and error in a dish, sparing their patients from the chemo side effects, lost time, and diminishing recovery chances.

Weiss and Tuveson hope to see the decades-old treatment concepts change within a few years. "Treating cancer should work similar to treating infections," Tuveson says. If you have a bad infection, the doctors may prescribe broad-spectrum antibiotics at first, but at the same time they also send your blood and urine tests to the lab and wait to see what bug you have. Once they know the exact culprit, they often change the meds to the most efficient antibiotic against it. "I'd like for the organoids to do the same—use them as a kind of a bacteriology test for cancer that will tell us the best way to kill it," Tuveson says. "I'm optimistic that they can point us to cancer's Achilles heel."

LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.

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Plants Fight for Their Lives

As arable land disappears, a genetic tweak might secure the world's food supply

BY SARA GOUDARZI

BIOLOGY + BEYOND | GENETICS

IT'S 2050. THE WORLD POPULATION has increased by 2.3 billion to 9.9 billion. Demand for food has risen 70 to 100 percent but a warming planet, extreme weather, and a decrease in arable land is threatening food security. Luckily, farmers can grow crops more densely, increasing yield from smaller plots of available agricultural land.

Packing crops so tightly wouldn't have been possible three decades earlier. That's because, despite looking docile, plants are actually hypercompetitive. Grow two plants too close together and they start competing for resources like minerals, water, nutrients, and—once they start to shade one another—sunlight. Without adequate light, plants adapt rapidly through what's called shade avoidance response (SAR). They reallocate energy into growing taller in an effort to harness sunlight, which results in stunted root growth and accelerated flowering time.

We're not trying to be Dr. Frankenstein. We've been modifying genomes for 10,000 years.

"This comes at a tremendous cost," explains Ullas Pedmale, an assistant professor at Cold Spring Harbor Laboratory, where his lab studies the interactions of plants and the environment. "This change in energy basically leads to lower crop and biomass yield. The plant is now like, 'Hey, I'm stressed, I've got very limited light, so let me make my offspring or seeds as soon as possible,' because now the plant is thinking about its Darwinian evolutionary pressure to increase reproduction as soon as possible."

Understanding SAR is especially important as major food crops—such as wheat, corn, potato, and tomato—are shade avoiders. But what if there was a way to grow plants densely without sacrificing yield? By learning about the genes involved in shade avoidance, Pedmale thinks he can shut down the plant's state of distress, and perhaps engineer plants that can access sunlight but not panic into flowering early and stunting root growth, thereby reducing yield.

Pedmale has been researching how plants perceive and modify their architecture in response to light. Specifically, he is studying a group of proteins called cryptochromes, what he calls one of the eyes of the plant. Cryptochromes, the only group of receptors common among animals and plants, sense changes in the availability of blue light. A reduction in blue light or red light with an increase in far-red light indicates that a plant is in the shade, prompting it to switch on genes that, among other responses, stunt root growth. Understanding cryptochromes and their interplay with these genes could be an important aspect of mediating these responses and the key to growing crops at higher densities.

For more than 50 years, scientists have been studying how stems and leaves respond to being in the shade. Several years ago, Pedmale, who was originally looking into a plant's above-ground architecture, realized that the roots are also an important aspect of SAR. As it turns out, when the shoots grow taller, the roots stop growing. Roots not only keep plants stable but also draw nutrition from the soil. Additionally, they are sources of food in crops like carrots and radishes.

Plant organs are divided into sources and sinks. Leaves, for example, whose main function is photosynthesis, are considered source organs: They provide and fix carbon. The carbon is then distributed throughout the plant. The roots are unable to fix their own carbon and are sink organs. "When a plant has enough resources, it can allocate resources into the storage, or sink, organ," explains Christian Fankhauser, a professor of biology and medicine at the Center for Integrative Genomics, University of Lausanne. However, "if a plant wants to grow taller stems to take over the neighbors, it's going to have to put more resources into the stem."

PAGES 48-49: BNP DESIGN STUDIO / SHUTTERSTOCK

The plant is like, "Hey, I'm stressed, I've got very limited light, and so let me make my offspring."

This, Pedmale explains, becomes a vicious cycle as plants need a proper root system to support the shoot. "One recent aspect we're studying is how the shoots communicate with the roots because roots are below ground," he says. The roots can't see sunlight but it's clear the shoots are relaying that message to the roots. Researchers want to understand what is happening so they can block that signal from traveling to the roots, allowing the roots to do their job and in turn letting the shoots perform their functions and deliver the needed crops, Pedmale adds.

Further, a weak root system can be detrimental to crops, as roots are acting as a physical anchor and keeping plants secure. "It makes [plants] more vulnerable because if you have too much wind and they fall over, they actually are not going to be harvestable," Fankhauser says.

Pedmale is trying to figure out what signals lead to stunted roots. His team compared the roots of tomato and *Arabidopsis*—the lab rat of plants—seedlings grown in light to the less developed roots of seedlings grown in the shade. They discovered that hundreds of genes plants use to respond to stress were switched on in the shade-grown plants, including dozens that encode proteins called WRKYs and regulate gene expression. To confirm those genes were responsible for the stunted roots, they engineered plants so that specific WRKY genes stayed highly active even in full sunlight. They found that the roots were stunted, similar to shade-grown plants.

"So now we have a proof of concept," says Pedmale. He believes that perhaps selectively disabling the genes that push plants into the shade-avoiding mode could change their reaction so the roots can keep growing.

Using gene editing methods like CRISPR/Cas9, the researchers can interrupt or inactivate a gene by making a very precise cleavage in the genome. They can also reprogram gene expression by applying certain chemicals, like a type of steroid, to the plants. In this way, they can figure out exactly what works to help shade avoiders overcome their state of distress when they sense a reduction in blue and red light. CRISPR, in fact, is not unlike natural evolution—just much, much faster. With CRISPR, scientists can influence the direction of evolution, enhancing good traits and weeding out those that aren't beneficial.

At the moment, Pedmale can't estimate a timeline of when the results of this work could be scaled up and used in agriculture. "It's hard to pin down, because [there are] so many things I can see go wrong," he says. "For example, it's possible that a gene responsible for shade avoidance response also performs other functions, [like] defending against pathogens." Knocking that gene out, therefore, might cause other issues.

Another obstacle, and one that Fankhauser thinks is a major one, is public acceptance and related policies, as many people remain afraid of certain types of technologies when it comes to their food. Never mind that gene editing of the food supply goes back for millennia.

"It takes all this talking to people to try to convince them that we are not trying to be Dr. Frankenstein," said Fankhauser, who noted we've been modifying the genome of animals and plants to fit our needs for the last 10,000 years. "Obviously, for the first 9,950 years or so, we had no idea about the genetic underpinnings of this enterprise: It was all kind of a random process. But now we do understand the genetic underpinnings [and] we can actually intervene in a much more directed, precise, way."

Pedmale believes his research can have far reaching implications: "I feel that any progress we make in this area has the ability to touch everyone on planet Earth, because everyone has to eat."

SARA GOUDARZI's work has appeared in *Scientific American*, *The New York Times*, and *National Geographic News*, among others. Her debut novel, *The Almond in the Apricot*, came out in February 2022. Find her at saragoudarzi.com and @Saragoud.

Why AI Needs a Genome

AI could learn and adapt like humans with algorithms that work like genes

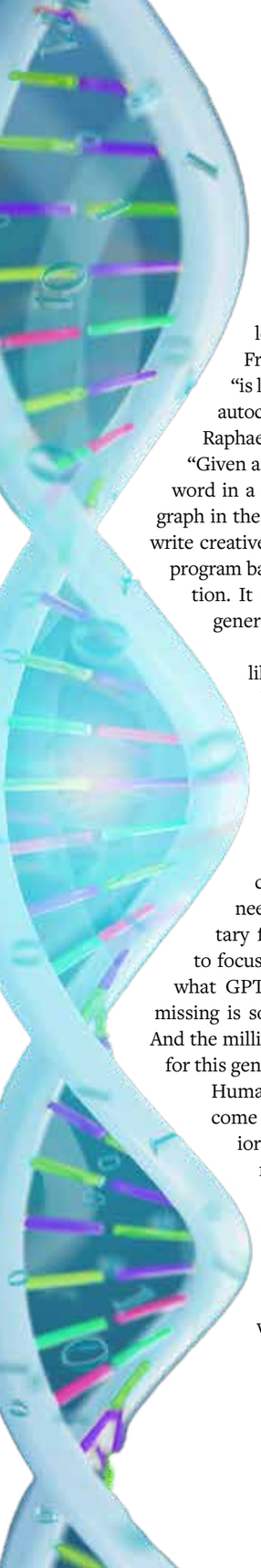
BY LINA ZELDOVICH

PRODUCTION PERIG / SHUTTERSTOCK

IT'S MONDAY MORNING of some week in 2050 and you're shuffling into your kitchen, drawn by the smell of fresh coffee C-3PO has brewed while he unloaded the dishwasher. "Here you go, Han Solo, I used the new flavor you bought yesterday," C-3PO tells you as he hands you the cup. You chuckle. C-3PO arrived barely a month ago and already has developed a wonderful sense of humor and even some snark.

He isn't the real C-3PO, of course—you just named him that because you're a vintage movie buff—but he's the latest NeuroCyber model that comes closest to how people think, talk, and acquire knowledge. He's no match to the original C-3PO's fluency in 6 million forms of communication, but he's got full linguistic mastery and can learn from humans like humans do—from observation and imitation, whether it's using sarcasm or sticking dishes into slots. Unlike the early models of such assistants like Siri or Alexa who could recognize commands and act upon them, NeuroCybers can evolve into intuitive assistants and companions. You make a mental note to get one for Grandma for the upcoming holiday season. She's been feeling lonely, so she could use company.

Let's make it clear—you're not getting this NeuroCyber C-3PO for Grandma this holiday season. Or any holiday season soon. But building such intelligent helpers and buddies may be possible if we engineer their learning abilities to be similar to those of humans or other animals, argues Anthony Zador, a professor of neurosciences at Cold Spring Harbor Laboratory who studies how brain circuitry gives rise to complex behaviors.



Currently, some AI models are making strides toward natural language processing using the so-called "deep learning approach," which tries to mimic how humans acquire knowledge. One such language learning model, GPT-3 by San Francisco-based company OpenAI, "is like a supercharged version of the autocomplete version on your phone," Raphael Milliere reported in *Nautilus*. "Given a definition, it can use a made-up word in a sentence. It can rewrite a paragraph in the style of a famous author. It can write creative fiction. Or generate code for a program based on a description of its function. It can even answer queries about general knowledge."

But to say that GPT-3 thinks like humans is an overstatement because it still makes funny mistakes no human would. In one of its articles, GPT-3 wrote that if one drinks a glass of cranberry juice with a spoonful of grape juice, one will die. "I'm very impressed by what GPT-3 can do," Zador says, but it still needs to be taught some rudimentary facts of life. "So it's important to focus on what it's still missing." And what GPT-3 and other AI programs are missing is something important: a genome. And the millions of years of evolution it took for this genome to form.

Humans and all other living beings come with a set of pre-wired behaviors written into their genes, accumulated along the long and circuitous evolutionary journey. Animals have been around for about 100 million years and humans for about 100,000 years. That's a lot of behavioral wisdom scripted in the genetic language of amino acids and nucleotides. On the contrary, AI comes with none. It has no genes to inherit and no genes to pass on. Humans and animals are products of both nature and nurture, but AI has only the latter. Without the former, it will never think like us.

That's why AI and robots are superior to humans only in specific things, while failing at others. They beat world class players in chess and Go. They outperform us in math and everything else that relies on rules or calculations. They are indispensable when things are dangerous, dull, too heavy, or too repetitive. But they aren't good at thinking on their feet, improvising, and interacting with the real world, especially when challenged by novel situations they weren't trained to deal with.

"I would love to have a robot load up dishes into my dishwasher, and I'd love to have a robot clean my house," says Zador, but we are far from making such helpful assistants. When it comes to household chores, we're at the level of Roomba. "You'd think stocking up dishes and tidying up a room is an easier problem than playing Go. But not for robots, not for AI." A child can learn to load up the dishwasher by watching their parent, but no Roomba can master the feat. In the famous match between 18-time world Go champion Lee Sedol and his AI opponent AlphaGo, the latter won by calculations, but couldn't physically place the pieces for its moves. It had to rely on its human assistant to move the stones.

Even if you train a robot to load the dishwasher with a set of dishes and cups, but then add pots and pans into the mix, you'd confuse your cyborg helper, while a child would likely figure out how to re-arrange the objects. That limitation is true across the AI world. "Currently, AIs are very narrow," says Blake Richards, an assistant professor at the School of Computer Science and the Montreal Neurological Institute at McGill University. Even the smart algorithms are limited in their tasks. "For example, a Facebook algorithm checks every image to make sure it's not pornographic or violent," says Richards—which is an impressive feat, but it can't do much else. "AIs are optimized for one specific task, but if you give them a different task, they are not good." They just don't learn like living things do.

In neuroscience, the term "learning" refers to a long-lasting change in behavior that is the result of

experience. However, most animals spend a small amount of time learning, yet they do well. "Most fish don't spend a lot of time learning from their parents and neither do insects, who are phenomenally successful in their behaviors," Zador points out. Bees are born knowing how to pollinate, flies expertly escape your swatter, and roaches scutter away at the sound of your walk. "But the vast majority of these behaviors are pre-programmed into a bee, a fly, and a roach," Zador says. "They don't learn these behaviors, they come out of the box—or out of the egg—with the ability to do whatever they are supposed to do." Where's the instruction set that enables them to do that? In their genome, of course. "Animals are enabled by the innate structures they were born with—their DNA encodes the instructions needed for them to execute these behaviors," Zador says.

Humans come with a lot of scripted behavioral wisdom. AI comes with none.

Mammals spend more time learning than insects, and humans devote a considerable span of years to acquiring knowledge and practicing their skills. "The amount of time we spend learning is at least an order of magnitude greater than other animals," Zador says. But we also come with a lot of "preprogrammed" wisdom. "Our ability to learn language is greatly facilitated by the neural circuits that are primed and ready to learn language." AI, on the contrary, isn't primed for anything, so it must learn everything from scratch.

Consequently, AI creators always had to do a lot of schooling. Up until the late 1990s, AI developers tried to give AI a set of rules to follow, says Richards. For example, when teaching computers to see, they would program them to recognize certain shapes and features. "Here are the shapes of eyes or noses, and if you see two eyes and a nose in between, that's likely a face," Richards explains. That unfortunately didn't work very well, because the world is simply too complex to fit into such rules. "We were not smart enough to hand-design these things for the messiness of the real world."

Today AI developers rely on three different types of learning. One is called supervised learning, in which an AI scans hundreds of thousands of pictures of, let say, puppies or elephants that are labeled as such—and learns how puppies and elephants look. But because humans don't stare at a stack of dog pictures to memorize what a dog looks like, it's not an ideal way to teach AI to think like humans, Zador notes. He cites one curious example. An AI system was trained on 10 million labeled images, which is how many seconds are in a year. Human children would have to ask a question every second of their life to process a comparable volume of labeled data; plus, most images children encounter aren't labeled. But children learn to recognize images just fine.

Another approach is unsupervised learning, in which AI trainers keep categories of elephants, puppies, cars, trees, and so on; the AI maps an image to a category and knows what it sees. That is more similar to how we do it—a human child playing with a little plastic toy dog and a big puffy stuffed one, will likely figure out they are the same animal.

And finally, the third way is reinforcement learning: The AI builders give it a goal—for example, find an object in a maze—and let it figure out how to do it. Rats are pretty good at finding cheeses in mazes. AIs still have a ways to go. That's because finding food is wired into the rats' genome, but AI does not have one. We are back to square one. To develop human-like intelligence—or rat-like intelligence to begin with—the AI needs a genome. But how do you give a set of genes to an algorithm?

Zador has an idea for that. Genomes encode blueprints for wiring up our nervous system. From that blueprint arises a human brain of about 100 billion neurons, each of which talks to about a thousand of its neighbors. "A genome is a very compact, condensed, and compressed form of information," Zador says. He likens a genome to CliffNotes—study guides that condense literary works into key plotlines and themes. "The genome has to compress all the key stuff into a form of biological CliffNotes," Zador says—and that's what we should try doing with the AI, too. We should give AI a bunch of behavioral CliffNotes, which it may then unfurl into a human-brain-like repository.

GO ON The artificial-intelligence version of Go, AlphaGo, may have beaten the game's human world champion, Lee Sedol, but AlphaGo couldn't physically place the pieces for its moves—a symbol of how far AI has to go before matching human intelligence.

SARAN_POROONG / SHUTTERSTOCK

Despite their biological complexity, genomes contain simplified sets of rules on how to wire the brains, Zador says. Living beings retain only the most important features for the most useful behaviors. Bees don't sing and flies don't dance because they don't need to. Humans can do both, but not fly. Zador is developing algorithms that function like a simple rule to generate behavior. "My algorithms would write these CliffNotes on how to solve a particular problem," he explains. "And then, the neural networks will use them to figure out which ones are useful—and incorporate those into its behavior." Later, more complex behaviors can be added—presumably all the way to the intelligent assistants who load dishwashers, pay bills, and converse with Grandma.

Another way to emulate learning, at least for simple organisms, is to equip an AI with their neuronal structure and let it advance by way of reinforcement learning. In one such effort, Nikhil Bhattasali, a student at Zador's lab, outfitted an AI with a subset of a digital

It took evolution 3.2 billion years to create Einstein. How long would it take AI?

mimic of a neuronal structure from a simple worm called *C. elegans* and let it learn how to move faster. "We took the wiring diagrams from *C. elegans* and essentially taught it to swim," Zador says. The worms perfected their squirming motions through millions of years of evolution. When equipped with only about two dozen neurons, the AI caught up with the swimming motions quickly. "With this built-in diagram, it learned to swim much faster than without."

Richards adds that the best way to let this AI develop would be to essentially mimic evolution. "Evolution endows us with innate capabilities, but evolution itself is not an intelligent designer," he notes. "Instead, evolution is a brute-force optimization algorithm. So rather than hardwiring any specific behavior into AI, we should optimize a system and then use that point of optimization for the next generation of AI—much as you do with evolution."

If these ideas work, will that combination of human intelligence and computer speed instantly propel the cyborg to the singularity, intelligence that surpasses our own? If it took 3.2 billion years to create a human Albert Einstein, how long would it take to create an AI equivalent of him?

Richards doesn't think AI will get to the Albert Einstein level, and here's why: It will likely hit the energy bottleneck. Making an AI amass as much knowledge as Albert Einstein, or even an average human, might require such an enormous amount of electrical power that it would be too polluting to sustain. The GPT-3 produced the equivalent of 552 metric tons of carbon dioxide during its training—the equivalent of 120 cars in a year—only to think that grape juice is poison.

"I think we're pretty far from the singularity," Zador chuckles. He cites a statistical reason why it's not worth worrying about. Suppose that machines do replace us as the next Einsteins," he theorizes. "By definition, very, very few of us make outlier genius contributions like Einstein. So does it matter if the probability of making such a contribution drops from 1 in 100 million per generation to basically zero?" Not really.

So building an AI equivalent to Albert Einstein may be neither worth the energy nor statistically important. Besides, the genius theoretician was known to be messy, spacey, and forgetful. With an AI like that, you'd be cleaning up after your assistant.

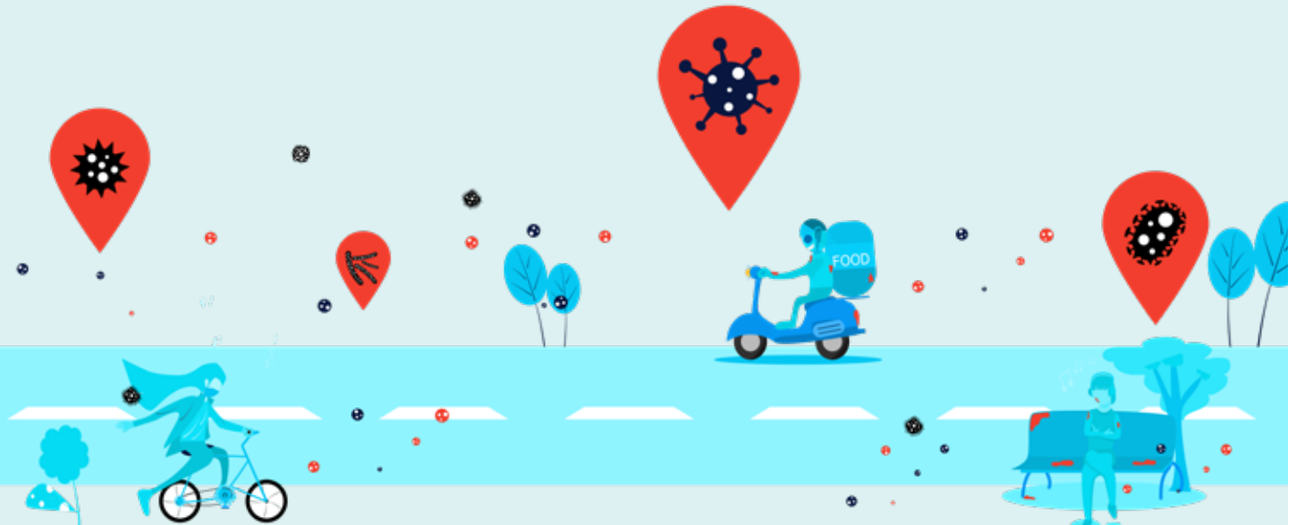
LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.

How Does Anyone Stay Healthy in a World Full of Germs?

Computational biology is uncovering the immune system's tricks for identifying foreign invaders

BY JOELLE RENSTROM

ALL IMMUNE SYSTEMS are not created equal. The evidence is all-too familiar: We all know that work colleague who never so much as sniffles, even when everyone else in the office is out sick. These days, immune system inequality plays out with especially grim consequences, as one person with COVID-19 dies, while another in a superficially similar state of health experiences only mild symptoms. But *why* our immune responses vary so widely—and how to make everyone's system more resilient—is one of the great mysteries in medicine.



JUNE VITA / SHUTTERSTOCK

BIOLOGY + BEYOND | HEALTH

The search for answers has led Hannah Meyer to a class of white blood cells known as T-cells, and to their point of origin in the thymus, a bean-shaped organ located near the heart. Meyer, bioinformatics expert and a fellow at Cold Spring Harbor Laboratory, notes that T-cells not only identify and eliminate cells infected with foreign invaders like SARS CoV-2, they also signal other parts of the immune system to mobilize when needed. Those functions depend on the ability of T-cells to reliably distinguish dangerous pathogens from the body's own cells, a skill that they acquire in the thymus … somehow. "We know the thymus represents everything," Meyer says, "but we don't know how it's regulated."

Within the thymus, T-cells and epithelial cells interact in virtually limitless combinations, sequences, and distinct micro-environments. It's possible to observe these interactions directly in a lab, but trying to explore them all empirically is like trying to anticipate the outcome of a chess game by trying out every possible move: overwhelming. Meyer is designing computer simulations that model the combinations virtually and determine which ones are more significant for the training of T-cells in the thymus. Those results can then allow much more tightly targeted laboratory studies.

Lab studies of immune cells and computer models of immune responses have often existed in separate academic realms. Meyer is now integrating the two, as part of a larger movement called computational biology. "We need experimental and computational approaches together to achieve a baseline understanding of what 'healthy' means and to understand what might have gone wrong in certain pathologies," she says.

In Meyer's lab, her team designs software to simulate conditions experienced by T-cells. Researchers can plug in environmental variables, assess the probabilities of various cellular responses, and develop experiments to compare the model's results with the behavior of actual immune cells. The goal is to figure out what it takes to turn a newborn T-cell into a focused disease fighter—and eventually, to map out the links between infectious disease, cancer, diet, and the immune system.

Why our immune responses vary so widely is one of the great mysteries in medicine.

THE JOURNEY OF THE T-CELLS begins during a person's childhood, when immature T-cells follow a trail of chemical signals that leads them to the thymus. T-cells are born in the bone marrow, initially equipped only with the necessary sensors (called receptors) to detect these chemical bread crumbs. Before they leave the thymus to fight disease, they must develop a whole additional set of specific receptors that allows them to identify every type of healthy cell, tissue, and protein; otherwise, they will end up attacking the wrong target. "T-cells have to learn about anything they could encounter elsewhere in the body, and they have to do it without traveling," Meyer says. Given that the average body contains 30 trillion cells of some 200 varieties, each T-cell needs an extremely thorough education.

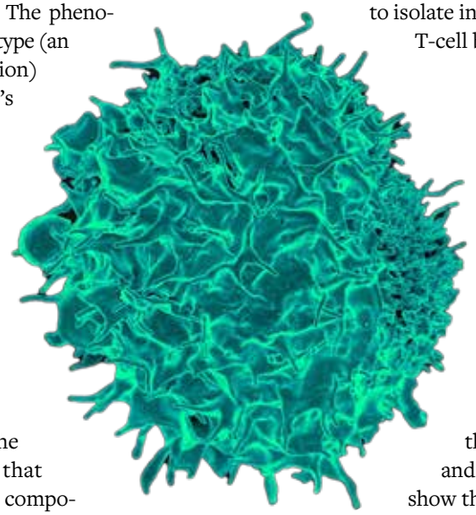
That education comes from specialized cells within the thymus, the epithelial cells. These cells have the ability to present small snippets (called epitopes) of all the proteins a T-cell could encounter throughout the body, thereby teaching T-cells the "look" of virtually any healthy cell and tissue. As a result, when T-cells finally head out of the thymus on their disease-fighting mission, they know that any cell with a foreign, unknown look to it must be sick and dangerous.

Researchers broadly know that thymic epithelial cells train T-cells by enabling them to see all protein epitopes of the healthy self, but they are lacking the specifics. They don't know how the training is scheduled, how many epithelial cells a T-cell has to visit before it is considered to have passed its education, or whether the T-cells must be exposed to the different epitopes in a specific sequence. Understanding such details might explain some of the key differences between effective and flawed immune responses.

In 2018, Meyer was working on a separate but similarly complex problem: What are the differences between a healthy heart and one that is prone to heart diseases? As with her current research, she turned to computer models and simulations to understand the key genetic and physiological components that make a heart beat efficiently. To help bridge the gap between experimental and model studies of the human heart, she developed a suite of computer programs, including the phenotype simulator. The phenotype simulator translates genotype (an organism's genetic information) into phenotype (the organism's observable traits). For example, brown hair is a phenotype that results from the genotype for hair pigmentation. The translational outputs can be quite complex, given the tremendous number of environmental factors and feedback mechanisms within the body that can influence phenotype.

Meyer is now applying the computational methods that helped elucidate the genetic components of heart physiology to her favorite puzzle in biology: the education of T-cells by thymic epithelial cells. In this case, her simulations allow researchers to plug in multiple important factors (such as the localization and duration of the education) and then see a range of likely immune-related characteristics that the cells might acquire in response. The resulting datasets provide what Meyer calls a "ground truth" about the ways that immune response differs across these simulated scenarios. By experimenting with different inputs, researchers can probe specific cellular interactions and relationships that affect immunity. Those results can then guide lab studies to search for associated molecular changes in the T-cells.

Meyer focuses specifically on interactions between T-cell and epithelial cells in the thymus, but her simulations are proving far more widely useful: T-cell interactions in different tissues and with different interaction partners are possible. For instance, studies show that autoimmune disease, in which the body attacks its own cells, is correlated with genes and environment, but the cause-and-effect relationship is undetermined. Meyer's simulation offers a way forward by allowing researchers to isolate individual processes influencing T-cell behavior.

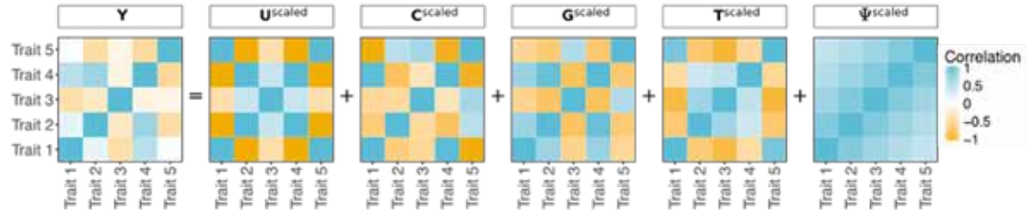


LITTLE FIGHTER A colorized electron micrograph of a T-cell.

MEYER'S COLLEAGUE Semir Beyaz, an immunology researcher at Cold Spring Harbor Laboratory, is particularly fascinated by using Meyer's computational methods to examine the link between cancer, diet, and the immune system. Studies show that high-fat diets contribute to greater tumor formation in mice, apparently by impairing the immune system's ability to weed out cancerous cells, but scientists don't know what processes associated with fat lead to weaker T-cells. There are so many variables and mechanisms at play that Beyaz calls it a "multidimensional data mess."

To cut through the clutter, Beyaz collaborates with Meyer to seek causal relationships between diet and cancer phenotypes that might have taken years to recognize without computer assistance. The computational analysis and simulation generate new hypotheses, which he can then use to devise an experiment and study whether that dietary factor triggered a response directly, or whether it is mitigated through immune cells such as T-cells. "As a bench scientist, you never know if something is actually regulating the process you're studying until you do functional experiments like turning the process off or over-activating it," Beyaz says.

NIAID



CUTTING THE CLUTTER Graphical representation of trait correlations, generated by Hannah Meyer's Phenotype Simulator.

Meanwhile, Meyer is building on her previous success in software development by developing ways to help experimental researchers like Beyaz to identify those kinds of processes more effectively. Her new software focuses on individual cellular interactions and "treats each cell as an independent agent," she says, allowing simulated immune cells to move naturally around a 3-D grid and keeping track of their interactions along the way. Meyer wants to find out if different routes, interactions, or sequences of interactions in the thymus alter a T-cell's immune functionality. The results will again suggest experimental follow-ups. If her models indicate that T-cells traveling on a certain course are more likely to fail and attack healthy cells, for example, researchers could investigate whether there is a preferred order of T-cell migration in the thymus, or if the cellular maturation process depends on location.

Genetics and immunology researchers around the world are already relying on Meyer's software for their work; her software suite for genetics analysis, including phenotype simulator, is freely available and has been downloaded more than 34,000 times. This established user group reports problems, which then helps Meyer develop revisions to fine-tune the simulator for specific research tasks. "Making other people's work easier is a big highlight," she says.

Meyer's software isn't about to reveal a miraculous cure-all that can boost the immune system on demand.

T-cells have to learn about anything they could encounter in the body, and they have to do it without traveling.

"People want a recipe to make COVID-19 go away, but that's not how science works," Beyaz says. "We don't even know the molecular and cellular basis of complex diseases. We need to understand those processes and how they interact." What Meyer can do—what she *is* doing—is provide the tools to figure out, at last, how those processes work. Slowly but surely, those tools are bringing us closer to the day when we can all be like that guy who forever beats the sniffles.

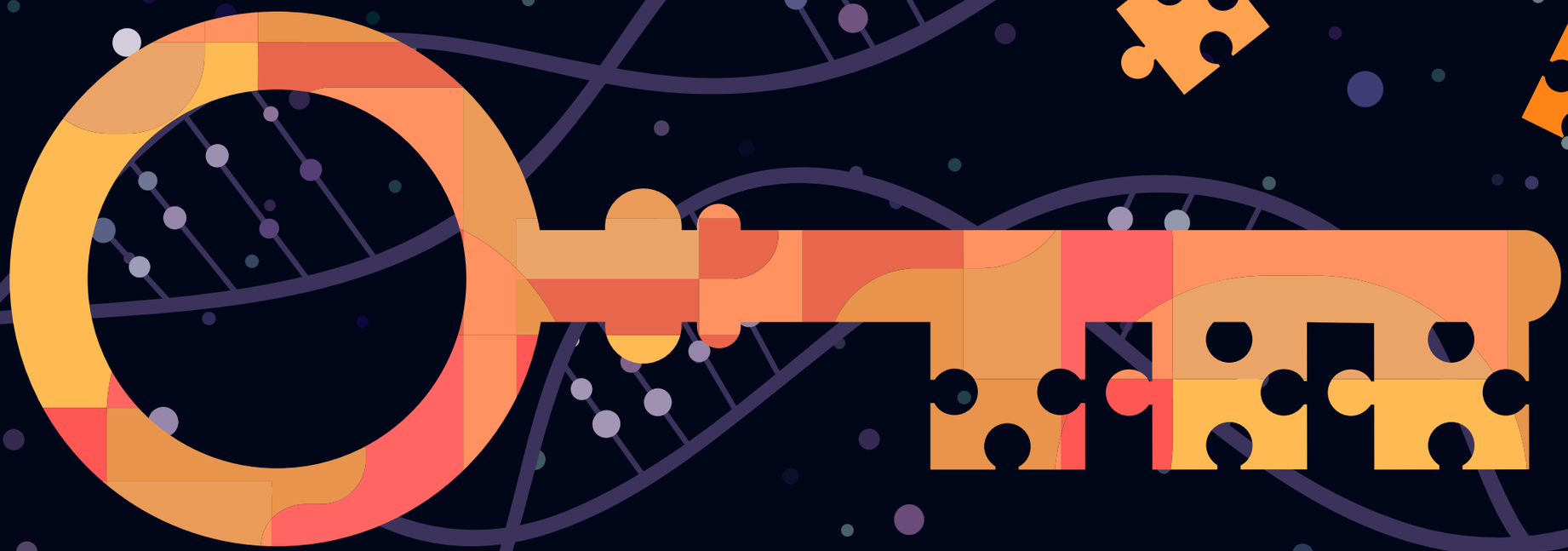
JOELLE RENSTROM is a science and tech freelancer whose work has appeared in *Aeon*, *Undark*, *New Scientist*, *Astrobiology*, *Slate*, and others.

HANNAH MEYER / CSHL

After 100 Years of Research, Autism Remains a Puzzle

One geneticist is determined to piece together the causes

BY LINA ZELDOVICH



MICHAEL WIGLER, A PROFESSOR at Cold Spring Harbor Laboratory was surprised. A molecular biologist and geneticist, with a background in mathematics and medicine, he devoted two decades of his research career to studying the causes of autism. In the early 2000s, Wigler and his team revealed that a certain portion of autism cases have genetic underpinnings. One of the team's goals was to elucidate the full extent of autism's genetic causes in order to find clues to its treatment and prevention. The team thought they had a good theory, which they dubbed the "unified hypothesis," but in 2017 that theory began to develop cracks. Now, the most recent findings produced by Wigler and his colleagues are not at all what they expected.

Based on theory, the team projected that affected siblings would share more genetic determinants inherited from their mothers than the fathers. But the findings showed the opposite. "In fact, we see a greater signal of sharing from the father than from the mother," Wigler says. That parental gender surprise is a head-scratcher that the team has only recently been able to explain. "It's a puzzle. And we do not like our solutions."

A complex condition, autism afflicts 1 in 44 children in the United States, according to the Centers for Disease Control. It manifests itself in a multitude of symptoms, from social awkwardness to anxiety, repetitive behaviors to resisting change. That variability is the reason why it's called an autism spectrum disorder, or ASD. Where on the spectrum an individual fits matters greatly. Those who fall into the high end of the spectrum have better prospects—they are the high-functioning individuals who often have special abilities such as superior math skills or photographic memory, which help them cope with life challenges, such as social anxieties. At the low end of the spectrum are those with intellectual disabilities and those who don't talk at all. It's estimated that 31 percent of children with ASD have an intellectual disability, and an even greater number have problems with motor skills.[1]

PAGES 62-63: MAGIC PICTURES / SHUTTERSTOCK

Gender has always been a big part of the conundrum. Boys are about four times more likely to be diagnosed with autism than girls. According to statistics published by the Johns Hopkins School of Public Health, 1 in 34 boys has autism (2.97 percent) compared to only 1 in 145 girls (0.69 percent). Girls often have different symptoms than boys. For example, they tend to suffer more from anxieties rather than display repetitive behaviors, and because anxieties can be masked or overlooked by clinicians, a certain percentage of girls may end up being misdiagnosed. Instead of being placed on the spectrum, some may be diagnosed with psychiatric disorders, such as depression or anxiety, says Catherine Lord, a practicing clinician who focuses on autism and is a professor of human development and psychology at the University of California, Los Angeles.

Some women self-diagnose later in life, having grown up without a clue that they might be autistic. Despite the increased focus on autism in the past two decades, which has lifted some of the social stigma from the condition, that trend is rising, Lord says. "The number of self-diagnosed people who are female is increasing every day," she says. "And it's not so much true for males." Brandy Schillace, editor in chief of *BMJ Medical Humanities*, author, and host of the Peculiar Book Club podcast self-diagnosed when she was an adult, after being told to "not be weird in public" for most of her childhood. "What makes autism a disability is not that you are broken, but that society disables you because it's not built around your needs," Schillace says.

It's hardly surprising that society doesn't cater well to people on the spectrum. After all, the exact definition of autism is still evolving. It took decades for society to even recognize autism properly as a condition. For a good chunk of the 20th century, autism was viewed as childhood schizophrenia. People with autism had been classified as psychopaths rather than neurodiverse individuals. Even the modern-day definition of autism as a spectrum disorder is still developing. The current, fifth edition of the Diagnostic and Statistical Manual of Mental Disorders, or DSM-5, described certain autistic features differently from its predecessor, the DSM-4, a testament to the fact that scientists are still working to identify its full nuances.

The underlying causes of the disorder had been equally puzzling, sometimes leading scholars in the wrong directions, such as pinning the blame on the children's mothers for their "cold and unemotional parenting." And without knowing what causes autism, physicians had—and still have—no means of preventing it or reducing its severity or risk of occurring. Just like with any other affliction, the path to mitigating autism lies in understanding where it comes from. If medics understand autism causes better, they would be able to design better therapies and potentially even better prevention.

That's exactly what Wigler has been doing for the past 20 years. As the genomic methods matured on the brink of the millennium, he hoped to find the answers in the genes of people on the spectrum. But piecing these answers together proved just as complicated and nonlinear as the history of this puzzling condition.

A COMPLEX HISTORY OF A COMPLEX DISORDER

The two people typically credited with the definition of autism are Hans Asperger, an Austrian physician who practiced in Vienna before and during World War II and Leo Kanner, a Jewish psychiatrist born in 1894 in Klekotiv, then in Poland and now in Ukraine, who later left Europe for the United States. Both began using the term "autism" in the 1940s. Asperger, for whom Asperger syndrome is named, described the children he studied as "autistic psychopaths," and as recent findings revealed, went on to collaborate with the Nazis on euthanizing patients that were deemed mentally unfit to exist in society.[2] Kanner's paper described his patients as not relating "in the ordinary way" to people or situations, and said their "behavior is governed by an anxiously obsessive desire for the maintenance of sameness." He was the one who, at first, explained the disorder by unemotional parenting, coining the term "refrigerator mother"—a view he denounced later.

It took decades for the autism research community to find out that neither man was the first to define and describe autism. A Ukrainian-born Jewish female psychologist named Grunya Sukhareva beat them by about 20 years. She was just well hidden behind the Iron Curtain.

Sukhareva graduated from medical school in Kyiv in 1915 and worked at the city's psychiatric hospital.

PROFOUND IMPRESSION Michael Wigler's interest in autism stemmed from his early life experiences. His girlfriend's brother was different from every other kid he knew. "He never looked you in the eye, but he knew every baseball player and all the statistics of the baseball players, and that's all he would talk about," Wigler says. "He made a profound impression on me."

LEN MARKS PHOTOGRAPHY

A few years later, she moved to Moscow, where she worked at the Pedagogical Sanatorium School for children with special needs. Some of them were traumatized by the dramatic events of the time—World War I, the Russian Revolution and civil war. Others were noticeably different from their peers: They had social deficits, motor-skills issues, preferred to play alone or interact with adults rather than kids their age. Sukhareva described one of the boys, a 12-year-old who read everything he could find and never played with toys, as an introvert "with an autistic inclination into himself."

Children with severe challenges sometimes lived in the sanatorium for two to three years, taking school classes and physical education, and receiving social- and motor-skills training. In a 1925 paper, Sukhareva described six boys with "autistic tendencies," chronicling their behaviors in finest details, including the fact that some were gifted—one excelled at playing violin and another had an incredible memory for numbers. Her findings, along with other records of the clinical work, were published in Russian and a year later in a German journal, where her name was misspelled as Ssucharewa.[3] The paper was not translated into

Gender is a big part of the conundrum. Boys are about four times more likely to be diagnosed with autism than girls.

English, so neither her term autistic nor her detailed observations reached the English-speaking psychiatrists. The German-speaking Asperger and Kanner might have read it, although it's hard to tell because neither one mentioned her name or referenced her in their own papers. The fact that the German version of her name had several typos likely didn't help either.

Nearly 100 years later, another Russian-speaking psychologist, Irina Manouilenko, found the original 1925 volume while working on her dissertation at the Karolinska Institute in Sweden. She decided to compare Sukhareva's descriptions with the modern-day standard definitions of the DSM-5, published by the American Psychiatric Association. She was surprised to see how spot-on Sukhareva's descriptions were. Manouilenko published these comparisons in a 2015 paper, "Sukhareva—Prior to Asperger and Kanner."[4] What the DSM-5 depicts as deficits in "social interactions" and understanding relationships, Sukhareva described as "flattened affective life," "lack of facial expressiveness and expressive movements," "tendency toward abstraction and schematization," and "keeping apart from their peers, avoiding communal games." And where the DSM-5 lists "stereotyped or repetitive motor movements," "insistence on sameness," "fixated interests," and "sensitivity to sensory input," Sukhareva's notes speak of "talking in stereotypic ways," being "pedantic," with "strong interests pursued exclusively," and sensitivity to noise or smell. Moreover, they were worded so simply that any parent or grandparent could understand them.

With the only diagnostic tools available to her being the keen power of observation, Sukhareva couldn't pin down the causes of this strange disorder. Instead, she focused on helping these children improve their social and motor skills by interacting with others and taking classes in painting, woodwork, and gymnastics—until they were ready to transfer to regular schools. Remarkably, the basic foundations of the interventions she set up haven't changed much over a century. Even some modern schools like Meristem, which prepare autistic young adults for an independent life and employment, in essence follow similar principles. Nonetheless, it took more than a few decades for diagnostic and analytical tools to mature enough for scientists to start chipping away at the puzzle.

COMBING THROUGH THE GENES

Wigler's interest in autism stemmed from his early life experiences. His girlfriend's brother was different from every other kid he knew. "He never looked you in the eye, but he knew every baseball player and all the statistics of the baseball players, and that's all he would talk about," Wigler says. "He made a profound impression on me."

Wigler didn't know the boy had autism. Later, in medical school, Wigler learned about the condition and became curious about its causes. At first, he doubted it was merely hereditary, attributing it to de novo mutations—not present in parents but arising in their child. "What struck me about this kid was that he was so different from anybody else in the family, so I thought from the beginning that autism was the result of a new mutation."

In the 1980s, he became interested in the so-called genome difference analysis. The method had proved useful in studying cancer causes so he decided to look for new mutations in the genomes of people with autism. At the time, such methods didn't yet exist, so his team published a paper describing the theory behind building such technology. Shortly after, at the end of the 1980s, Russian scientist Nikolai Lisitsyn, who had been working on a similar problem, contacted Wigler's lab. Lisitsyn had managed to solve a particular technical problem, and, detecting his interest in fleeing a collapsing Soviet state, Wigler invited him over to work at CSHL. This collaboration laid the foundation for the future methods of genomic analysis Wigler's lab would follow for years to come.

In the early 2000s, Wigler's team got a chance to put their genome mining tools to the test. Child psychiatrist Susan Folstein, at the time at Tufts New England Medical Center, had put together the first small simplex collection of about 200 families with instances of autism. She passed her collection to James S. Sutcliffe, an associate professor of psychiatry at Vanderbilt University who studies the genetic underpinnings of autism spectrum disorders. Wigler, who was looking for such collections, got in touch. After applying their genomic analysis methods to the samples, Wigler and his colleagues Lakshmi Muthuswamy and Jonathan Sebat showed that de novo mutations most certainly play a role.

The particular culprits were the so-called "copy number variations"—genetic glitches in which large chunks of the genome get deleted or duplicated. They looked as if someone tore a piece of the DNA string. "Your genome is a long string. It's a ribbon. And if you were to cut out a piece of the ribbon and then tie it together, you'd be missing a large piece of your ribbon," Wigler explains. "Some of them are really large, hard to miss with our new techniques, as easy as seeing a crater on the moon with a low power telescope."

Notably, not all de novo mutations cause trouble. There are about 100 to 200 de novo mutations in the genome for every birth, Wigler explains, and most of them don't affect anything. For example, genes involved in the sense of smell, or the immune system function vary greatly between people and some variants may even be advantageous. "There's great tolerance, and probably even positive selection for variation in these genes," he explains. But other genes' functions are so essential and specific that any changes would render the organism inviable or severely disadvantaged. The large copy number mutations are of that type, just too massive to leave no mark. And they were clearly more abundant in children that had autism than in their neurotypical peers. The team showed that the copy number variations were responsible for a substantial number of autism cases. That finding opened new horizons, sparking hope that by sequencing the DNA of people with autism, and looking for smaller sequence variants that alter gene function, researchers would be able to pin down the causative genes.

Previously, and still today, some scientists used more standard approaches, combing through collections for variants that might be common to people with similar disorders, a technique called Genome Wide Association Studies. One such collection was called AGRE, for Autism Genetic Resource Exchange, an effort aimed to shed more light on familial autism, studying the multiplex families—those that had multiple siblings and multiple members affected. Another collection named the Autism Genome Project was composed of different groups across the U.S. and Europe and gathered information about affected siblings. "The idea was to see what regions of the genome are shared between affected individuals across large collections," says Sutcliffe. "And that effort failed to find loci strongly influencing autism incidence," Wigler notes. "A very important failure, since it pointed to other causes, including new mutations."

The simple hypothesis that the mothers passed a strong autism-causing variant didn't stand the test.

Consequently, the Simons Foundation followed the lead of Folstein, and created the Simons Simplex Collection, or SSC. The effort was led by Lord, an expert in diagnostic criteria. They amassed genetic samples from 2,600 families, where only one child affected was on the spectrum while no siblings or parents were. "With SSC there was hope to find the 'core autism'—the mutation or set of genes specific to autism," says Sutcliffe. By comparing the autistic child's genome to the genomes of other family members, scientists aimed to pinpoint the genes that made this child different. Wigler worked closely with Lord to mine the SSC collection for answers and identify the genetic mishaps in children from families that did not have any other members affected. More recently, embracing the ideology of "strength in numbers," the Simons Foundation launched SPARK, an ambitious ongoing effort to collect data from 50,000 families of any kind and, so far, has collected about 30,000.

Notably and somewhat strikingly, de novo mutations played out differently in boys versus girls. While boys with "comparable-size craters" succumbed to autism, the girls did not. "It took a larger hit to make a girl autistic than it took to make a boy autistic," Wigler says. "That was one of the first indications that girls were resistant." In their 2011 study, Wigler and his team explained that women have greater resistance to autism from genetic causes.[5] The phenomenon became known as a female protective effect. "Female protective effect means that you need a higher load of mutations to rise to a phenotypically recognized Autism Spectrum Disorder that can be clinically determined," Sutcliffe says.

Yet, as seminal as the findings were, overall they explained only about 30 percent of autism cases. Neither was it possible to identify that definitive "core autism" set of genes. "We had hoped that there was one, but the answer is 'no, not really'," Sutcliffe says. "Today's data doesn't allow us to do that."

Aiming to solve the rest of the puzzle, Wigler's team proposed the so-called unified hypothesis of autism. They postulated that there must be other kinds of mutations that weren't possible to see so easily. For example, these mutations may not be occurring together in the genome but are instead spread out across multiple genes in different places. They also hypothesized that these mutations would be transmitted largely from the mother because—while mothers were better protected as females—they still could carry the autism-causing variants and then pass them onto their children. "We made a unified hypothesis, stating that autism results from de novo mutations and is transmitted by the survivors of the de novo, namely the girls," Wigler says.

When there were eventually enough large sample collections, with enough genome data, Wigler, in collaboration with Matt Wroten and Ivan Iossifov, also of Cold Spring Harbor Laboratory, and Kenny Ye

Healthy pregnancies are a good start to reducing autism severity and risk.

from Albert Einstein College of Medicine, developed a method to put their hypothesis to test. Specifically, the team wanted to measure the genomic differences between siblings that were discordant for autism (meaning one has it and the other doesn't) and concordant for autism (meaning both siblings have it). Overall, they applied the method to about 1,300 pairs of concordant siblings and 4,500 pairs of discordant siblings from the SSC, AGRE, and SPARK collections.

But the simple hypothesis that the mothers passed a strong autism-causing variant didn't stand the test. "Surprisingly, we observed that the concordant siblings shared more of the fathers' genomes." Wigler says. "The more extensive sharing of paternal than maternal genomes contradicted our expectations that mothers will be the primary source of damaging variants in the high-risk multiplex families." And that meant that there was more work to do: more hypotheses to formulate and prove.

Some hypotheses invoke complex genetics, and these might be correct, but Wigler and colleagues doubt it will be the full story. "The data is very hard to fit with standard genetic models," he says. They already have some other ideas that might help explain the mystery. He and another Cold Spring Harbor Laboratory researcher, Tobias Janowitz, considered theories that the mother's immune system plays a role, potentially impairing fetal development. Recent research supports such a theory. A 2017 study by Johns Hopkins Bloomberg School of Public Health team concluded that when pregnant mothers have fevers, their children's risk of autism increases.[6] A 2018 study by Columbia University researchers found that if a pregnant woman suffers a high fever in her second trimester, her child's chances of developing autism increase by 40 percent.[7]

The immune system can play an even greater role in the mother-father conundrum, Wigler thinks. For example, the father may be carrying an antigen—meaning a protein—that the mother's body doesn't like so it attacks it in the fetus. "The father may not be carrying an autism risk gene—just an antigen that the mother doesn't like," Wigler explains. "The kids who have autism, may be kids who've suffered from an immunological attack on them while they were in utero. Some portion of autism might not be caused by conventional genetics, but by the maternal fetal conflict."

While some genetic causes of autism, like the copy number variations, have become proven culprits, others will take longer to identify. A full list of autism's genetic underpinnings might take years, if not decades, to put together, and it still may be incomplete. In the meantime, Wigler says, healthy pregnancies are a good start to reducing autism severity and risk. That involves parents and physicians. Doctors can monitor pregnancies better. They can intervene earlier. They can watch out for certain dangerous conditions that occur in pregnancies, such as preeclampsia—a problem related to the placenta. "There isn't much we can do about genetic mutations yet, but we can work on the maternal-fetal conflict," Wigler says. "If we could control that process better, we could have healthier babies and reduce autism risk."

LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.

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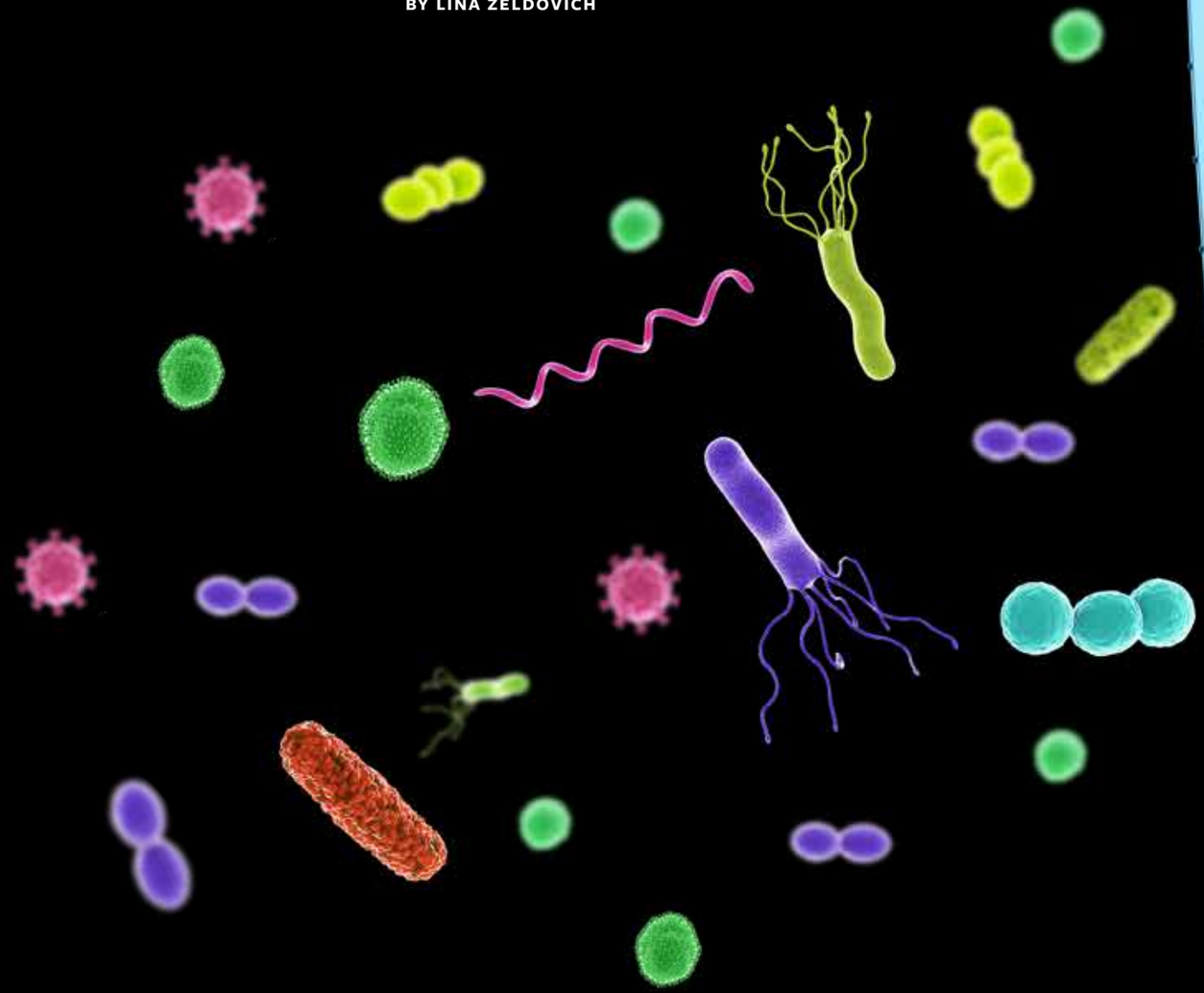
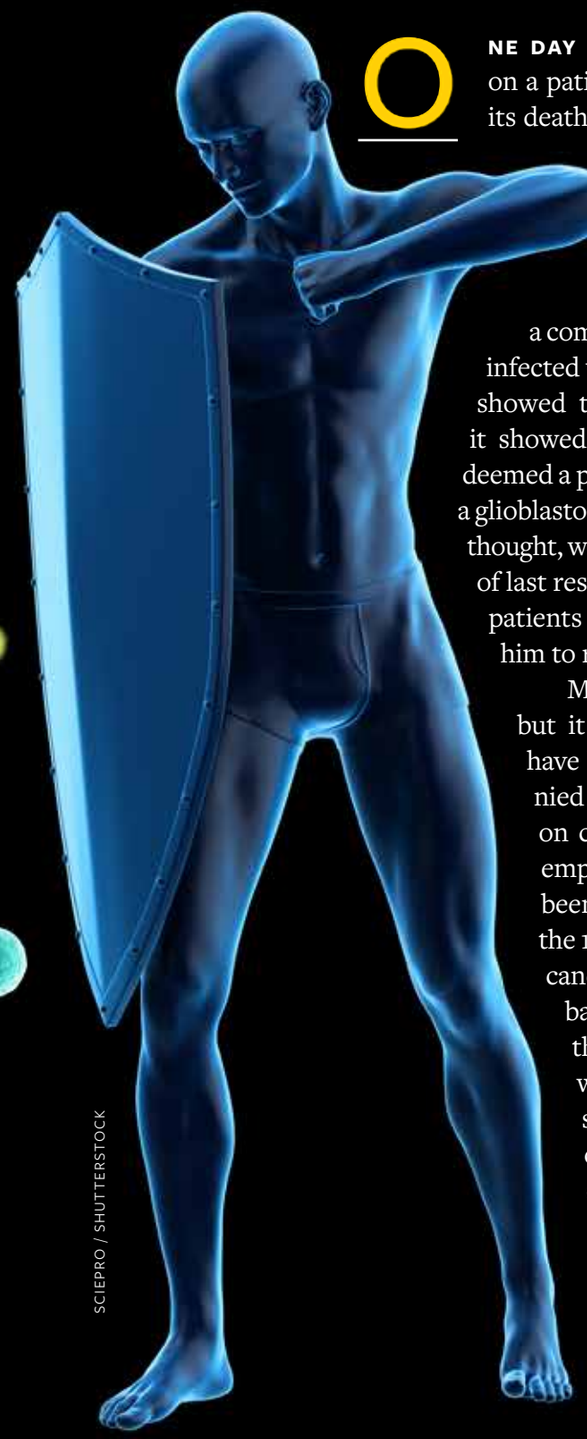
Triggering the Body's Defenses to Fight Cancer

Experiments once considered crazy are now helping scientists attack tumors

BY LINA ZELDOVICH





SCIEPRO / SHUTTERSTOCK

ONE DAY IN 2010, when oncologist Paul Muizelaar operated on a patient with glioblastoma—a brain tumor infamous for its deathly toll—he did something shocking. First, he cut the skull open and carved out as much of the tumor as he could. But before he replaced the piece of skull to close the wound, he soaked it in a solution containing *Enterobacter aerogenes*,[1] bacteria found in feces. For the next month, the patient lay in a coma in an intensive care unit battling the bacteria he was infected with—and then one day a scan of his brain no longer showed the distinctive signature of glioblastoma. Instead, it showed an abscess, which, given the situation, Muizelaar deemed a positive development. "A brain abscess can be treated, a glioblastoma cannot," he later told the *New Yorker*. Trying it, he thought, was worth the chance. He had done this only as a means of last resort in a couple of hopeless cases—but ultimately, his patients still passed away, which led to a scandal that forced him to retire.

Muizelaar's approach may sound beyond outrageous, but it wasn't entirely crazy. For over 200 years medics have known that infections, particularly those accompanied by fevers, can have a strange and shocking effect on cancers: Sometimes they wipe the tumors out. The empirical evidence for these hard-to-believe cures has been documented in medical literature, dating back to the 1700s. In the 19th century, some doctors tried treating cancer patients by deliberately infecting them with live bacterial pathogens. Sometimes it worked, sometimes the patients died. Injecting people with dead bacteria worked better and, in fact, saved lives, at least in some cancers. The problem was that it didn't work consistently and repeatedly so it never became an established treatment paradigm. Moreover, no one could explain how the method worked and what it did. Doctors speculated that infections somehow revved up the body's defenses, but even in the early 20th century, they had no means of elucidating the mysterious force that devoured the tumor.

Today we know that this mystery lies in the complex interplay of cancers and the immune system, says Mikala Egeblad at Cold Spring Harbor Laboratory, who studies the tumultuous interactions between cancers and the organisms they grow in. We know that cancers have an uncanny ability to pull the wool over the eyes of the immune system's cells—not only by hiding from them, but even co-opting them to help themselves flourish. "Tumors are dysregulated organs," says Egeblad—and they dysregulate the environment around them too. They cause a lot of turmoil and havoc wherever they take hold. Called the tumor microenvironment, that "battleground" is teeming with various microscopic players that cancers corrupt into unwitting allies.

"Our immune system is trying to protect us from various threats, including cancer," says Karin Pelka at Gladstone-UCSF Institute of Genomic Immunology who studies the cellular interactions that shape immune responses. "But cancer mutates in ways that it evolves to evade the immune system. So there's a constant battle going on."

In these dysregulated, messy ecosystems, infections may indeed serve as a force that rights the wrongs. They could reboot the body's normal defense mechanisms, making the immune system see the enemy. However, deliberately infecting cancer patients with bacterial pathogens faces a major obstacle. It will never pass FDA approval because subjecting people—who are already gravely ill and fighting for their lives—to yet another health threat is unethical, reckless, and risky. And yet new directions in cancer treatment draw on the immune system kickstart idea, albeit in a different way. Moreover, some of the immunological approaches to cancer treatments have graduated from clinical trials to actual clinics.

Today, medicine has better methods for resetting the body's idle defenses that don't involve infecting patients with pathogens. And there are different ways to do it, says Pelka. One of them employs the so-called oncolytic viruses—genetically engineered or naturally existing viruses that infect only tumor cells, multiply inside, then burst them open, invading more cells. Scientists are also trying to boost the immune system activity with specific cytokines—molecules that cells use to communicate with each other. An especially successful class of drugs now used against different types of cancers is called checkpoint inhibitors; it works by unleashing the body's warrior T-cells to kill cancer cells. Another strategy that has proven successful against some blood cancers are CAR-Ts (Chimeric Antigen Receptor T-cells), in which T-cells are taken from the blood, engineered in a lab to seek out the specific cancer—and then infused into the patient.

Revving up the immune system defenses is also much gentler on patients than the traditional methods like chemotherapy, which inevitably damage healthy cells, too. "The immunotherapy is much less toxic than chemotherapy," says oncologist Sylvia Adams, who treats cancer patients at NYU Langone Health System. "It doesn't change the patient's quality of life." It just "coaches" the immune system to tackle the tumor. And that's what oncologists are aiming to tap into.

He had done this only as a means of last resort in a couple of hopeless cases.

"We hope to train the immune system to recognize the tumor," Pelka says. This training could have a long-lasting effect because the immune system has a memory. Once the chemo is stopped, the cancer can regrow if not every single cell is killed. But if you train the immune cells to recognize the enemy, they will remember it. "This memory function is something that cancer immunologists are very excited about," Pelka says.

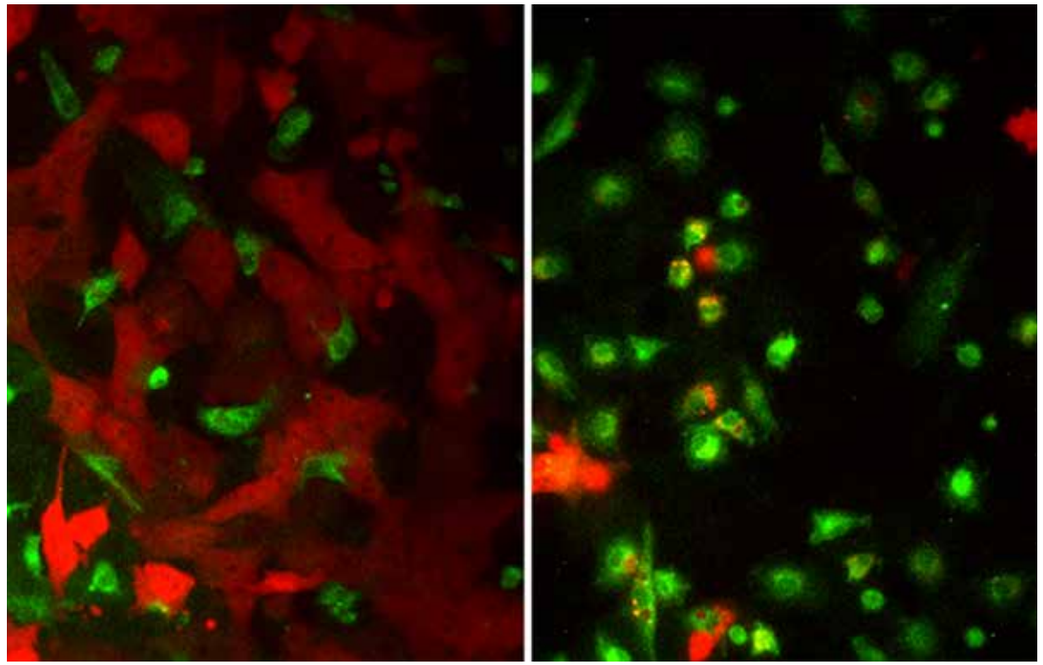
This immune system "training" works on the molecular level, and that's what cancer immunologists are investigating right now. Egeblad does it in a *Fantastic Voyage* style—by watching what the cells in the tumor microenvironment do. It is a bit like parachuting into the tumor trenches where the armies of cellular soldiers engage in military actions, sometimes deceiving each other, sometimes waking each other up from their molecular stupor. Egeblad is experimenting with a once-promising treatment that had fallen into disfavor because it also involved dangerous bacterial pathogens. First tried by a clever clinician over 100 years ago, it may be finally due for its 21st-century upgrade.

WILLIAM COLEY AND HIS TOXINS

In the fall of 1890, Elisabeth Dashiell, an athletic 17-year-old lady who was a close friend of John D. Rockefeller, Jr., came back from an adventurous trip to Alaska with a swollen hand, which she had hurt in a seemingly minor accident. Her hand was healing so poorly that she went to see William Coley, a young but prominent doctor, at the Memorial Hospital in New York, which later would become the Memorial Sloan-Kettering Cancer Center.[2]

It turned out Dashiell's hand wasn't healing at all—Coley diagnosed her with an aggressive round cell sarcoma, a type of bone cancer.[3] Coley operated, but it did little to help—Dashiell died from the metastases 10 weeks later. Her cancer was so rapid and vicious that it left a profound impression on Coley. He embarked on a quest for better options.

While scouting medical literature, Coley found the seven-years-old medical records of a patient who had round cell sarcoma on his neck, which kept growing back despite five surgeries. The man, a German immigrant named Fred Stein, was considered inoperable and hopeless, until he contacted erysipelas—a skin infection caused by streptococcal bacteria that manifests itself in fever and large, red patches on the face and legs. The infection, which spread over his neck and face, produced a



RESCUE REPROGRAMMING Tumor-associated macrophages (green) can help cancer cells (red) grow, but they can also be reprogrammed to kill them. The image on the left is a petri dish with mouse tumor-associated macrophages and breast tumor cells 48 hours after mixing the cells together; in this case, the macrophages don't kill the cancer cells. But when CSHL postdoc Lijuan Sun added interferon-gamma and MPLA (an immune booster) to the petri dish, the macrophages were reprogrammed and began eating the tumor cells. The image on the right shows the results 48 hours later.

COURTESY OF CSHL

strange side effect—his tumor all but vanished. According to the records, Stein went home in good health. Coley searched the Lower Manhattan tenements for Stein, and found him still alive and well, with no signs of cancer.

As he continued plowing through medical literature, he found that various prominent medics also had observed curative effects of infections on cancer. For example, English surgeon Sir James Paget noted that infection may cause a regression in some tumors. In 1867, German physician Busch reported a case similar to Stein's, in which a tumor disappeared when the patient developed erysipelas. And in 1888, only two years before Dashiell died, another medic named Bruns intentionally gave a cancer patient a shot of streptococcus to induce erysipelas, after which the tumor shrunk. Altogether, Coley read about over 40 cases documenting the beneficial effects of infections on tumors.

Coley tried injecting three patients with streptococcal bacteria. The tumors seemed to shrink, but two of the patients died from the infection, so Coley switched

The teams showed the combo suppresses tumor growth and metastasis.

to using dead bacteria—killed by heat. He also added another "cooked" bacteria into his concoction, *Serratia marcescens*, which, when alive, can cause infections of the respiratory and urinary tracts. He used the combo, which was dubbed Coley's Toxins, on inoperable sarcoma patients with a fair amount of success—it was better than anything else available at the time. For the next three decades, Coley's Toxins were widely used—until radiation and chemotherapy techniques came of age. These newer treatments soon surpassed the dead bacteria in popularity and Coley's Toxins were all but buried in the annals of medicine.

Coley's Toxins had several problems. Medics like predictable and repeatable results, and the bugs—alive or dead—were finicky subjects. Coley made 13 different preparations of the toxins, with some more effective than others. Sometimes he administered them intravenously, sometimes intramuscularly and in other cases he injected them directly into the tumors.[2] Many doctors who used his toxins didn't get the same results. Moreover, no one, not even Coley, could elucidate how the toxins worked.

Part of the issue was that Coley's method was essentially ahead of its time. In the early 20th century scientists didn't have the means to take a *Fantastic Voyage* trip into the tumors' den. They couldn't peek at the tumor microenvironment. They didn't know that cancers can corrupt and co-opt the immune system cells. And yet, Coley was on the right track, Adams says. "When we, oncologists, talk about cancer immunology, we always refer to Coley as the person who had the first inkling into the power of the immune system."

Today, scientists have much better tools to watch these battles in action. They can literally see the toxins flipping the immune cells' tumor-tackling switches back on.

INTO THE TUMOR'S TRENCHES

If you could indeed journey into the tumor trenches, you'd likely find the place very crowded. Tumors like to surround themselves with all kinds of normal, healthy cells, which they corrupt and co-opt into helpers.

In a healthy environment these cells would be performing their designated activities, Egeblad explains. Fibroblasts would be building scaffolding for various tissues to grow, such as muscle or bone. Pericytes would be making blood vessels. The immune system warriors B-cells and T-cells would be scouting for perpetrators, releasing antibodies, and killing the sickly cells—those infected by pathogens or mutated. Neutrophils would join the fight by ingesting invading microorganisms and releasing enzymes

that kill them. And macrophages would clean up all the cellular debris and zap various rogue cells with nitric oxide—a toxic, free radical molecule they spew out. Many of these cells also interact with each other through molecular messaging. T-cells stimulate B-cells to secrete more antibodies. Macrophages activate T-cells to sic them on the agents of disease. In response, T-cells activate macrophages by spitting out inflammatory cytokines, molecules that regulate the body's response to disease and infection. All these different players keep each other alert and engaged, a well-working biological defense team.

Normally, all these activities are supposed to spot mutated cells and wipe them out before they proliferate. But if and when a mutated cancerous cell—which may divide into two, or four, or a little clump—manages to avoid detection, they break the normal order of things. They start issuing their own molecular messages that confuse the cellular team. Tumors corrupt fibroblasts, which, in turn, turn off some of the T-cells and B-cells, essentially making them blind to the cancers' presence. Tumors can "reprogram" macrophages—they secrete molecules that attract these cells, but instead of devouring the mutants, macrophages release growth stimulants for them. "So the immune system can provide the tumors with growth factors, which benefit the cancer," says Pelka.

He injected three patients with streptococcal bacteria. The tumors seemed to shrink.

Scientists call such molecular "turncoats" tumor-associated macrophages, or TAMs. These TAMs do more damage than just feeding the tumors—they turn off T-cells and B-cells, so they no longer see the enemy. Moreover, these TAMs start taking cancer around the body, enabling metastases to take hold. They actively help malignant cells hitch rides in the bloodstream, traveling far and wide and settling in new locations. "Data strongly suggests that TAMs help tumor cells in and out of blood vessels—they are physically nurturing cancer cells," says Egeblad. "So even though the immune system has the ability to recognize the cancer cells, it gets turned off. The cells get suppressed." Cancers indeed pull the wool over the immune system's cellular army. The cells need an eye-opener.

For Egeblad such an "eye-opener" was an experiment one of her postdoctoral researchers did a few years ago. He was trying to make TAMs go back to their normal feisty state and start killing glioblastoma. He mixed a bunch of cancer cells and TAMs in a petri dish, and then he added some "magic dust"—a mix of bacteria-derived toxin and another immune-boosting compound called interferon gamma. A part of the innate immune system, interferons are proteins that mediate the body's defense responses, and the gamma type is specifically known for its anti-cancer activity.

The toxin-interferon combo packed a punch. The blinded macrophages "woke up" and attacked cancer—a battle that the postdoc captured on camera. "It makes macrophages speak in a different way to the T-cells," explains Egeblad. "They change the signals they are sending out, and these new signals make T–cells effective in recognizing the cancer cells. But we think it also likely works on all other cells, too. It changes the entire environment."

Egeblad and Adams teamed up to investigate how this combo would work on tumor cells taken from real patients. Adam's team collected lung fluids from patients with breast cancer that had metastasized to the lungs and transported them to CSHL. Egeblad's team extracted tumor cells and immune cells from the samples, treated them with the toxin-interferon combo and watched the immune cells waking up to the cancer's presence. "We were able to turn them on to the tumor cells in the dish," Adams says. "We reprogrammed them to kill the tumor." In a recent study, the two teams showed that the toxin-interferon combo also suppresses tumor growth and metastasis in breast and ovarian cancer in mice.[4] They hope to eventually try this in humans, too.

Understanding the tumor microenvironment has other potentials. It might help answer exactly how cancers first "set up shop," corrupting immune system cells and making them work for themselves. When metastatic cancers first arrive to a new location, that location isn't set up to nourish them, Egeblad says. All the body's cells there are healthy and doing their regular job—and yet, the cancer manages to corrupt them again. Too often patients go home seemingly cured from their cancers, only to discover that it metastasized someplace else, or even many places, and is already in advanced form. "We'd like to understand how metastases develop, what enables cancer cells to succeed in the new organ or how it gets eliminated by the immune system there," Egeblad says. "Once we figure that out, we'll be able to put an end to cancers' spread."

LINA ZELDOVICH grew up in a family of Russian scientists, listening to bedtime stories about volcanoes, black holes, and intrepid explorers. She has written for *The New York Times*, *Scientific American*, *Reader's Digest*, and *Audubon Magazine*, among other publications, and won four awards for covering the science of poop. Her book, *The Other Dark Matter: The Science and Business of Turning Waste into Wealth*, was published in 2021 by Chicago University Press. You can find her at LinaZeldovich.com and @LinaZeldovich.

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Alexei Koulakov

The physicist turned neuroscientist on Dr. Deep Nose—an artificial intelligence apparatus that one day will be able to diagnose diseases by smell

INTERVIEW BY LINA ZELDOVICH

ON HEALTH AND ODOR

Recent research finds that many diseases, including cancer, tuberculosis, and Parkinson's, can manifest themselves through volatile compounds that change a person's scent. Our bodies release certain metabolites—products of our metabolic activities. Some of these molecules are volatiles and become part of our scent, or "odorprint." When we become sick or start developing a disease, our metabolic processes start functioning differently, emitting different volatile molecules or mixtures of them, so our odorprint changes too. For example, patients with Parkinson's disease produce an unusually high amount of sebum, a waxy lipid-rich biofluid excreted by the sebaceous glands of the skin, which sensitive noses can detect.

ON HUMAN NOSES

Humans aren't as proficient at smelling as some other animals because we have a very small subset of the olfactory receptors, which allow us to discern a very limited number of scents. Our primate ancestors had about 850 olfactory receptor types, but we retained only 350 functioning ones. The rest of them simply don't work. They are the remnants of our former glory. Still, when used in various combinations, these receptors allow us to smell an astronomical amount of odors, just not as many as other animals. Dogs have about 850 receptor types and mice about 1,100 or 1,200, so they are capable of discerning a much greater variety of smells—including those produced by the malfunctions of our bodies.

WHY DIAGNOSE VIA SMELL?

If you look at history, this idea isn't revolutionary. Hippocrates, Galenus, Avicenna, and other physicians of ancient times used their noses as diagnostic tools. A wound with a nasty smell could mean it was infected. And bad breath signaled a host of ailments. Today, physicians don't sniff their patients because we have a slew of various diagnostic tools, but many of them are invasive, unpleasant, painful, or expensive. Meanwhile, we can grab these informational molecules literally from thin air within seconds.

DEVELOPING DR. DEEP NOSE

We think that diseases will likely manifest themselves by the presence of multiple volatile molecules—a cocktail of them. So we are using mice—who are much better than us at smelling—to help us identify and catalog various scents. We are collecting the info about what neurons activate in response to specific smells in mouse brains. Once we have mapped out that olfactome, we can start training our Dr. Deep Nose on all those smells. And once it's trained, this electronic artificial intelligence network will be able to diagnose or identify you—because a scent also uniquely identifies a person. We are hoping that our Dr. Deep Nose will essentially revolutionize the diagnostics system. It won't happen within the next 5 years, but we can see it happening within a couple of decades. So in 2050, your doctor's visit might quite literally be a breeze.

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