HARBOR TRANSCRIPT

Cold Spring Harbor Laboratory

Emma walks!

The first step was research

VOLUME 36 · ISSUE 2 · 2016

Cold Spring Harbor Laboratory

PRESIDENT'S MESSAGE

I am proud to share with you the exciting news that fundamental research that began at Cold Spring Harbor 40 years ago is now improving the lives of children with a deadly disease called Spinal Muscular Atrophy (SMA). You can read the feature about how Nobel Prize-winning CSHL scientists discovered a new way that genes are expressed and how Professor Adrian Krainer continued this work to eventually discover a drug that is having a major impact on treating a devastating, lethal childhood genetic disease. Such work is what CSHL is all about—investing in the best and the brightest scientists early in their careers and letting them pursue basic discovery science in earnest, without the encumbrances of more traditional academic environments.

The SMA drug, nusinersen, (to be marketed as SPINRAZA), was developed in Krainer's lab in collaboration with a biotech company. That story illuminates the Laboratory's ability to move nimbly from basic to applied research as opportunities arise. The application of knowledge and technologies created at CSHL to therapeutics and diagnostics is a growing strength of our institution. The Center for Therapeutics Research (CTR) that we announced this year, with an initial $25 million in infrastructure funds provided by New York State, will allow CSHL to continue to seize clinical opportunities that examine the links between cancer, nutrition and metabolism, as well as the role of the brain in diseases like obesity and diabetes.

Yes, the brain and its peripheral nerve system is a factor in cancer and many other diseases. So I hope you will read the article about Tony Zador's better, cheaper, faster approach to mapping the brain. Understanding connectivity and how the brain and the periphery is wired will help explain the mysteries of behavior and also improve our ability to diagnose and treat disorders of the brain and other parts of the body.

We must continue to support basic research and I hope that this is recognized by the new leadership at the federal, state and local levels to ensure the brightest future possible. With public and private support, Cold Spring Harbor Laboratory promises to pursue with utmost resolve its dual mission of leadership in biological research and science education. Life science is the key to better lives for us all.

Harbor Transcript

Volume 36, Issue 2, 2016

Harbor Transcript is published by the Department of Public Affairs of Cold Spring Harbor Laboratory.

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On the cover and on the web:
Emma Larson, 3, is one of the first kids whose life has been transformed by a drug treatment for spinal muscular atrophy whose origins we trace to basic research at CSHL.

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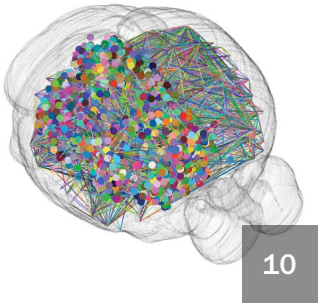
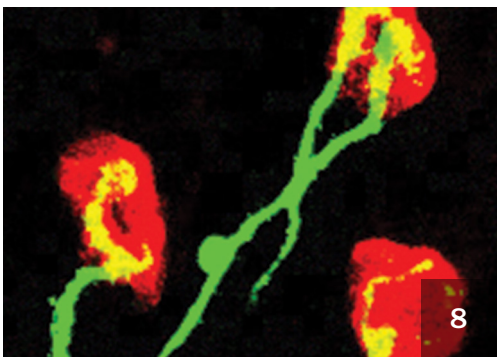




FEATURES

FACULTY & FRIENDS

Life-saving SMA drug is a triumph

Adrian Krainer speaks of a moment in his scientific career when something unexpected placed everything he'd previously done in sharp relief. "It was 1999, and I was attending a workshop at the National Institute of Neurological Diseases and Stroke (NINDS)," says the CSHL Professor and recent inductee into the National Academy of Arts and Sciences. "The meeting was about spinal muscular atrophy, an illness I knew almost nothing about."

SMA, he would learn, is a motor-neuron disease, "not unlike Lou Gehrig's disease (ALS), except that SMA tends to affect babies and young children. It's the leading genetic cause of infant mortality."

"You have progressive loss of motor neurons. As these neurons degenerate, the muscles they activate weaken and atrophy. This impairs one's ability to move—in young children, the ability to sit up, eat, even breathe." Infants with the most serious form of SMA, called type 1, often don't survive to their 2nd birthday.

By 1999 Krainer was already widely respected for research he describes as "a relentless pursuit of RNA splicing." Splicing is an activity that takes place continuously in the nucleus of cells: the editing of RNA messages copied from the DNA of genes. Each edited message serves as a template for the manufacture of a specific protein, one of the many thousands our lives depend upon.

At the NIH workshop, Krainer instantly understood that his research might help identify the molecular basis of the splicing problem in SMA, and ways to fix it. In patients, a gene called SMN1 is either missing or doesn't function. The gene encodes a protein called "survival of motor neuron" (SMN) that motor neurons need to flourish. Humans have a nearly identical backup gene called SMN2, but due to a mutation, its RNA copy does not splice properly. As little as 10 percent of the critical SMN protein is made in patients with severe SMA, not nearly enough to support normal neuromuscular development.

Krainer began to investigate the splicing error in SMN2. Today, 17 years later, a drug based on a molecule his lab designed and tested in mice has had impressive successes in late-stage clinical trials, dramatically improving the lives of babies and children with SMA. Biogen and Ionis Pharmaceuticals, commercial developers of the drug, called nusinersen, have filed for approval with the FDA.

This is the story behind the story of nusinersen. It's about how basic research produced the Nobel Prize-winning discovery of RNA splicing, and later, knowledge about its complex machinery, which then provided a basis for efforts to invent a therapy for SMA. Scientists at Cold Spring Harbor Laboratory play prominent roles throughout, illuminating how basic research brings benefits to society. The story line takes us back to the declaration of the War on Cancer by President Nixon in 1970, which spawned research leading to the discovery of RNA splicing.

Discoveries 'neither planned nor expected'

Almost simultaneously in 1977, a number of teams that included Richard Roberts [above, left] and Louise Chow at CSHL and another at MIT led by CSHL alumnus Phillip Sharp [above, right], discovered RNA splicing. James Watson called it "a once-in-a-lifetime event that completely transformed all of biology." Roberts and Sharp won a Nobel Prize for the work in 1993.

Yet its full impact did not register until after the human genome was sequenced 25 years later. Scientists then learned that human beings have about 21,000 genes, a number shockingly small considered against the vast number of distinct proteins these genes encode. This diversity is possible because the RNA copies of most human genes are spliced in different ways to generate different proteins, something called alternative splicing.

> "The pursuit of fundamental knowledge makes possible discoveries and breakthroughs that can't be anticipated..."
>
> *Bruce Stillman*

Splicing thus helps explain the evolution of biological complexity and one of the means by which organisms adapt. The other great implication of Roberts' and Sharp's discovery pertains to illness. Not just SMA but a vast number of human maladies, from muscular dystrophy to breast cancer, sometimes can be caused by errors in the splicing process.

CSHL figures prominently in the story of splicing's discovery and the development of the first drug therapy to correct a splicing error, for two key reasons, says President Bruce Stillman: "Our bedrock commitment to basic research, and our strategy of investing in talented young scientists and then giving them room to run."

Nusinersen was invented at CSHL because the Laboratory had long been committed to basic splicing research. "Importantly," Stillman stresses, "that outcome was neither planned nor expected, much as the discovery of splicing itself had not been years earlier. The pursuit of fundamental knowledge makes possible discoveries and breakthroughs that can't be anticipated, and it is often these that lead to spectacular practical advances like new life-saving drugs."

Rich Roberts, who since leaving CSHL in 1992 as Assistant Director has been Chief Scientific Officer at New England Biolabs, explained that "all this early splicing research was going on at Cold Spring Harbor because a decision had been made to study tumor viruses—and *that* was because we were going to study cancer."

Jim Watson, after taking the helm at CSHL in 1968, responded to Nixon's War on Cancer with a commitment to studying viruses, because viruses were genetically simple and some were known to cause cancer. They offered a way of studying how genes are expressed and how the proteins they encode can change a normal cell into a cancer cell. Among Watson's early decisions was to hire dozens of the

Adrian Krainer embarked on splicing research as the first CSHL Fellow.

smartest young scientists he could find. Among them were Sharp and Roberts, who arrived in 1971 and '72, respectively.

Sharp and Roberts were given the freedom to follow where their research took them. Neither had the faintest notion they would discover RNA splicing. Roberts proposed to use a newly discovered class of proteins called restriction enzymes to cut the gigantic DNA molecule into small bits that could then be sequenced, if slowly, using a manual method. Working separately, Sharp had already used the first such enzymes to map and sequence parts of a viral genome.

Both men applied their skills to a basic mystery that molecular biology was then tackling: When an activated gene's "message" is copied into RNA—that first step toward making a protein—how does the RNA message actually form? Their conclusion was that genes were "split." They were not copied directly into the form of a protein-encoding RNA. Rather, a preliminary, raw RNA copy of the gene was edited, or spliced. [illustration, facing page]

A young talent hand-picked to explore

"Splicing was discovered when I was a freshman in college," Adrian Krainer remembers. "It opened the door to a whole series of questions that motivated me. How does this splicing process happen? What is the machinery? We had no idea."

Most newly minted Ph.D.s in biology go on to serve as postdoctoral researchers in other labs and are obliged to shift their research focus. An innovative program at CSHL enabled Krainer to continue splicing research he had begun at Harvard under his Ph.D. advisor, Tom Maniatis. In 1986 he was named a CSH Fellow, the first in a distinguished line that includes Nobel laureate Carol Greider. He was hand-picked by Rich Roberts, who remembered being impressed with a talk Krainer had given at the 1984 CSHL meeting on RNA Processing.

Krainer, with Roberts as a mentor, was able in the late 1980s to pursue "frontier" questions. He would use an experimental system in which cells are broken open and their contents sifted to understand the components required for splicing. At Harvard, he had devised such a "cell-free system" to study splicing in a test tube. At CSHL he could add and subtract various "fractions" from the cell nucleus, where the splicing reaction occurs, to isolate the individual components needed to make splicing happen.

Krainer recalls: "My first real breakthrough at CSHL was to take one of these fractions [a cell extract that spliced RNA] and purify a single protein out of it, which is now called SRSF1." It has proven to be one of the most important of the 200-odd proteins now known to be involved in splicing. In July 1990 he published two key papers: one characterizing SRSF1 as a factor that binds to RNA and that must be present if splicing is to occur; the other reporting that its concentration influences *alternative* splicing, the phenomenon that accounts for the ability of a single gene to encode different proteins.

For a full explanation of RNA splicing watch our cartoon: http://bit.ly/RNAsplicing To see how the SMA drug works: https://youtu.be/YLIuIVwg_y4

Krainer and postdoc Akiya Mayeda soon made a second major discovery: They identified the function of another regulatory splicing factor, an RNA-binding protein with the unwieldy name hnRNPA1. Curiously, it had an antagonistic effect on SRSF1 when the splicing machinery was faced with choosing between two competing splice-sites. The site ultimately chosen for the cut depended on which of the two proteins was more prevalent. They later understood that SRSF1 acts as a splicing activator, and hnRNPA1 as a splicing repressor.

Krainer's team applied what they'd learned in cell-free systems to the much more complex environment of living cells. In dozens of papers written over a decade, they took apart and reassembled various parts of the splicing machinery and factors that encouraged and impeded it. They were fleshing out the complex workings and regulation of the phenomenon that Sharp and Roberts discovered in 1977.

When Krainer attended the NIH workshop on SMA in 1999, he was working on a problem called exon skipping in the messenger RNA of a gene called BRCA1. Various mutations in BRCA1 are associated with heightened ovarian and breast cancer risk. Krainer was studying a rare BRCA1 mutation in which the change of a single DNA letter caused the gene's RNA copy to splice incorrectly.

"The NIH workshop was a watershed moment for me because in SMA the splicing error in SMN2 is so obviously similar to the error we were studying in BRCA1," Krainer

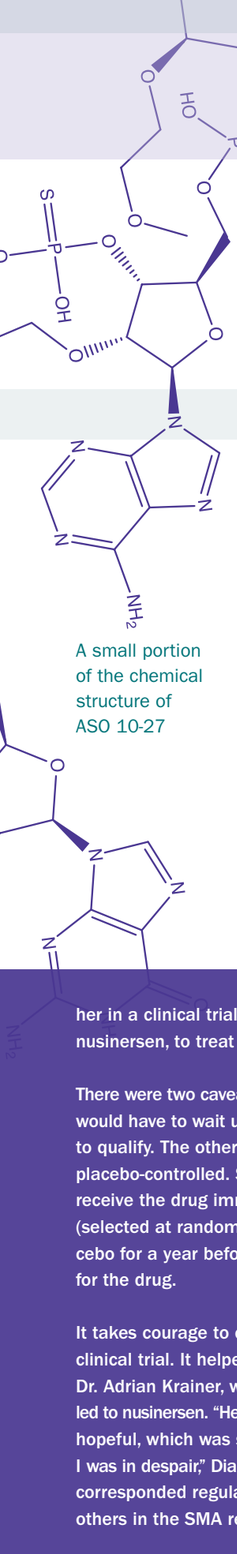
"She's my little fighter"

"You feel like the rug is ripped out from under you with this disease."

That's how Dianne Larson, the mother of an SMA-affected child, describes the experience of finding out. The problem in most cases, including that of her daughter, Emma, is that when the illness begins, "there are no signs whatsoever."

She learned to sit up and to crawl, like other toddlers her age. At Emma's 12-month pediatrician checkup, her mom remembers: "She was still moving her legs. Still bearing weight on them. They said, 'She's great, she's perfect. Take her home.' But then, at 13 months, all hell broke loose. All of a sudden she just wasn't moving her legs, and I'm like, 'What the heck happened?' It really took her overnight—that's what it felt like."

Emma was diagnosed with type 2 SMA, meaning her cells could make a small amount of usable "survival of motor neuron" (SMN) protein, but not enough to carry her very far in life. She was examined by Professor Darryl C. De Vivo, M.D., founding director of the Pediatric Neuromuscular Disease Center at Columbia. He explains that in SMA "like with so many other diseases, usually you have to lose about 50% of function before it becomes clinically apparent." In other words, Emma was losing motor neurons even as she met her first milestones. Now she was symptomatic—but there was a ray of hope. Dr. De Vivo suggested enrolling



A small portion of the chemical structure of ASO 10-27

says. The 18th exon in BRCA1 was skipped just as the 7th exon was in SMN2, in both cases with harmful effects. "The BRCA1 mutation was very rare, but now suddenly here's a more common disease I'm learning about in which all the patients have the same defect. It was readily apparent that if we could find a solution, it would apply to all SMA patients."

Toward a drug for SMA

Krainer's initial work on SMA was very basic. But within 3 years, he and postdoc Luca Cartegni had figured out a way to encourage the "inclusion" of exon 7 in SMN2's messenger RNA.

Before getting to that point, Krainer had the remarkable surprise of demonstrating that the splicing factor SRSF1—his first major scientific discovery at CSHL—was central to the pathology causing exon 7 to be skipped. His earlier work now placed him in position to understand why SRSF1 was not binding to the RNA, and why the 7th exon was being skipped over.

In 2003, Krainer and Cartegni tested an idea "that Luca proposed: we could make a synthetic molecule to bind to exon 7 and act as a surrogate for the [SRSF1] protein that was no longer binding there." Krainer and Cartegni invented a method called ESSENCE that promoted inclusion of missing exons in SMN2 and BRCA1.

This exciting result was noted by Dr. Frank Bennett, a leader of drug development at Isis Pharmaceuticals (since renamed Ionis Pharmaceuticals). "We reached out to Adrian, to see if there was an opportunity to collaborate. It was like two streams of basic research coming together," Bennett says.

Ionis had performed research to establish the first commercial platform for antisense drugs. In 2004, Ionis agreed to license ESSENCE from CSHL. Krainer and Bennett coordinated efforts to optimize the system. "Working with Adrian has been one of my most enjoyable collaborations," Bennett says. "It was almost magic the way things ended up working."

There were more surprises along the way. After Krainer's identification of SRSF1 as the missing splicing activator that caused exon 7 to be skipped, he realized that the corrective molecule—made up of two parts—was also able to correct exon skipping when stripped down to a short, "naked" sequence of RNA, called an antisense oligonucleotide, or ASO. This was key in the design of the drug now called nusinersen. Efforts began to synthesize an ASO with the greatest ability to promote inclusion of exon 7.

Another surprise was finding that it was possible to prevent exon 7 skipping by placing ASOs at various positions on either side of exon 7, not within it—in other words, within the "gibberish" segments, or introns, that were soon to be spliced out. The most promising of these, ASO 10-27, was chosen by Ionis for clinical development.

Krainer's team was determined to find out why the ASO worked so well. Here was their next surprise. The molecule, they discovered, attached to the RNA at a position normally occupied by a *repressor* of splicing. That repressor turned out to be hnRNPA1—the existence of which was the second major discovery of Krainer's CSHL career. "There's no reason in the world that both SRSF1 and hnRNPA1 would come into play in our much later SMA work," Krainer acknowledges. "Pure serendipity!" says Rich Roberts, who has experienced similar luck in his own illustrious career.

Yimin Hua was the Krainer lab postdoc who conducted the "screen" that identified ASO 10-27 and performed the crucial last phase of the preliminary work on the candidate drug, carrying it forward into animals. In April 2008, a paper by the team reported that ASO 10-27 injected into a mouse model of type 3 SMA corrected SMN2 splicing in motor neurons in the spinal cord and eliminated related pathology. Subsequent papers showed that the drug reversed symptoms of severe, type 1 SMA in mice.

Ionis received FDA permission to begin clinical trials in 2011. By early 2015 pivotal phase 3 trials were in progress, and in August, one of those trials, in infants, was ended early. The drug was effective enough in the company's eyes to justify providing it to all enrolled infants. In the fall, a new drug application was submitted to the FDA, seeking approval for the drug that began its life as ASO 10-27 in the Krainer lab.

"What all of this basic research has led to," sums up Roberts, "is the very first practical application in the clinic of our original discovery of splicing back in 1977. People have always asked me: 'Why was your splicing discovery important?' Well, now I can point to something everyone can appreciate. The nice thing is, if we can do it for this disease of splicing, we can do the same for others, too."

Peter Tarr

her in a clinical trial for a new drug, called nusinersen, to treat SMA.

There were two caveats. One was that Emma would have to wait until her second birthday to qualify. The other was that the trial was placebo-controlled. Some children would receive the drug immediately while others (selected at random) would receive placebo for a year before being eligible for the drug.

It takes courage to commit a child to a clinical trial. It helped to ask questions of Dr. Adrian Krainer, whose research at CSHL led to nusinersen. "He was very sweet and very hopeful, which was so important because I was in despair," Dianne says. She has since corresponded regularly with Krainer and others in the SMA research community.

Emma was enrolled in the trial but had to wait 6 months. "All during that time she was going through a swift regression. She got to a point where she wasn't able to sit up anymore and hold her bottle."

Her parents took her to Dr. De Vivo on her second birthday. "We were not waiting," Dianne says. She kept a diary:

First injection given March 3, 2015.
Second injection, end of March.
Third injection scheduled for May.

"It was after the second shot, but before the third," Dianne remembers. "I was in the bedroom; Emma was in the den. Now mind you, she can't move more than a few feet. All of a sudden, I hear her voice, getting closer and closer to me. What has she done? '*Emma?*' I say. Next thing I know, she's right beside me on the bedroom floor, right by the door. I was freaking out! I couldn't believe she had crawled all the way from the den."

Dianne's diary for **May 2015**: "*Something amazing is happening. Emma is regaining strength and endurance to crawl longer distances. She's also asking to stand and walk.*"

In June, Emma took her first steps, leaning against an ottoman. By September she took her first steps in a walker.

"She's my little fighter," Dianne says. "One of the little soldiers who's part of this battle to treat SMA or hopefully wipe it out."

By August of 2016 Emma was learning to use crutches to walk, and getting ready to begin preschool. Dr. De Vivo, whose team at Columbia's SMA Clinical Research Center in late 2012 administered the first dose of nusinersen ever given to a sick child, is thrilled to observe the results.

The impact of nusinersen has been "absolutely transformational," in De Vivo's view. The impact goes beyond SMA, he says, "to the whole field of rare diseases, particularly those that affect the developing nervous system and emerge postnatally."

Peter Tarr

One experiment

Think of this pair of images as the scientific equivalent of an exclamation point. Here is proof positive of an idea first advanced in 2003 by CSHL Professor Adrian Krainer and colleagues. In a paper entitled “Correction of disease-associated exon skipping,” they suggested a way to make a drug to treat spinal muscular atrophy. A neuromuscular disease, SMA is the leading genetic cause of infant death. But hopefully for not much longer.

Caused by the failure of a gene called “survival of motor neuron 1” (SMN1), the illness results from the lack of a protein—called SMN—that developing motor neurons need to thrive. A backup gene called SMN2 is of limited help to patients lacking SMN1, owing to a mutation that limits the amount of functional SMN protein it can generate. At the root of this error is the way the SMN2 gene’s RNA—its template for making a protein—gets spliced, or edited, before being dispatched to cellular protein factories called ribosomes.

Krainer has spent his 30-year career at CSHL meticulously assembling a picture of how splicing works and how it goes awry in diseases like SMA. From 2003, when these images were published, through 2011, his lab tested hundreds of tiny molecules called antisense oligonucleotides (ASOs), designed to fix the SMN2 splicing error. On the left, in a mouse genetically engineered to have severe SMA pathology, we see neuromuscular junctions—where motor neurons plug into muscle fibers. Note the reduced “arborization” in the axon terminals (green) at the endplates (red) where the axons form synapses with the muscle fibers. On the right, a triumph: a view of how such pathology is corrected at junctions in mice modeling severe SMA after being treated with an ASO drug called nusinersen that was developed by Krainer’s team in collaboration with Ionis Pharmaceuticals.

Peter Tarr

Anthony Zador

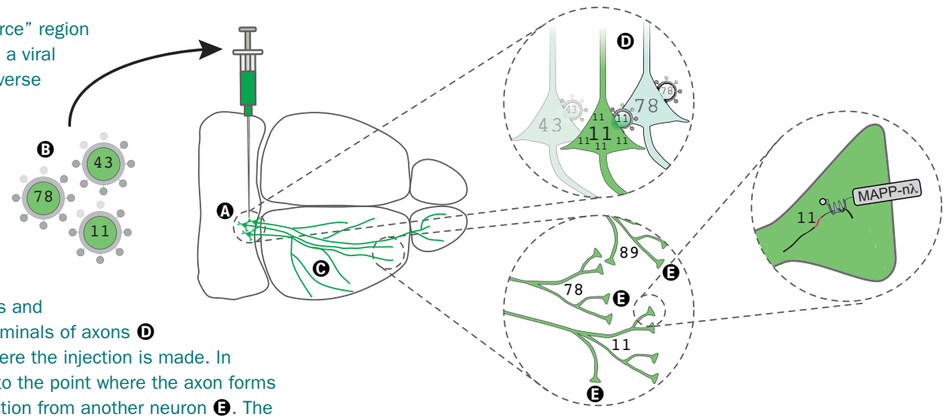
The signals travel along a network, sometimes connecting, other times going their own way, passing like unconnected thoughts in a cerebral night. A fabulously complex network of neurons that carries this information somehow represents everything in our minds, from our thoughts to our perceptions to our plans for action.

Anthony Zador, M.D., Ph.D., the Program Chair of Neuroscience and the Alle Davis Harrison Professor of Biology at Cold Spring Harbor Laboratory, would like to know how we go from what's in our heads to what's in our thoughts.

His overarching goal, he says, is to "understand how neural circuits form the basis for behavior." He wants to use the grey matter in his own brain to grasp how people "go from a circuit of 100 billion neurons, to complex behaviors, and, ultimately, to subjective experience."

He recently took an important step toward tracking the connections between those neurons, brain-wide, when a technology he has developed with several graduate students over the last 4 years bore fruit. Other researchers have gone to great lengths to see where individual neurons are located, using techniques like electron microscopy to map these fundamental units of the brain. While effective, these high-resolution approaches are laborious, expensive, and focus on tiny sections of the brain [facing page].

Zador's new approach [below] is revolutionary, mapping the brain not optically but with the help of gene sequencing technology. He has invented a way to hitch unique barcode-like identifiers to individual neurons. Those barcodes, made of long strings of RNA "letters," not only tag brain cells distinctively but also travel throughout the length of each cell's thread-like axonal projections, riding these all the way to the synapses where they connect with projections sent by other neurons. Using RNA sequencing, Zador can map the route through the brain that each of these axons travels.



An injection into a "source" region Ⓐ of the brain contains a viral library Ⓑ encoding a diverse collection of barcode sequences, which are hitched to an engineered protein that is designed to carry the barcode along axonal pathways Ⓒ. The barcode RNA is expressed at high levels and transported into the terminals of axons Ⓓ in the source region where the injection is made. In each neuron, it travels to the point where the axon forms a synapse with a projection from another neuron Ⓔ. The barcodes are sequenced when the brain is later dissected.

His team performed a proof of principle in the locus coeruleus (LC), a small group of neurons located in the brain stem. In a single experiment, they were able to trace axons from hundreds of LC neurons to their destinations in the cerebral cortex. The same technique can be used to trace projections from any neuron in the brain to any other region. The method's most immediate application—and advantage—is to trace the destinations of hundreds, thousands, even millions of neurons at a time, in a single, inexpensive and rapidly performed experiment.

Marrying molecular biology & neuroscience

Zador's talent and creativity has been recognized both in and out of science. He has received generous support from the Allen Brain Institute, and was selected in the inaugural class of Allen Distinguished Investigators. In 2015, he received an unexpected honor from *Foreign Policy* magazine, which named him one of 100 Leading Global Thinkers.

While marrying molecular biology and neuroscience is enabling Zador to tackle important questions about the brain, by his own admission he was an unlikely candidate to develop such an approach. "It's pretty ironic that I would undertake this," he acknowledges. In his earlier training, he had focused on developing other skills, and paid little attention to molecular biology.

He studied physics and linguistics at UC Berkeley, then went on to earn Ph.D. and M.D. degrees at Yale while conducting research in theoretical neuroscience and neural networks. During his medical training, he considered becoming a neurosurgeon before dedicating himself to research.

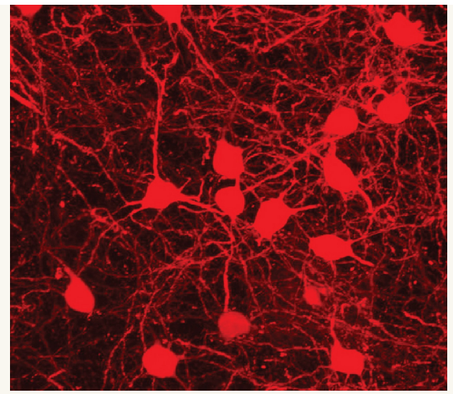
Zador conducted postdoctoral research at the Salk Institute in La Jolla. Arriving at CSHL in 1999, he put his lab to work on how the brain "computes"—how, for example, neurons represent sounds and how the processed signals are harnessed by other parts of the brain as a basis for acting and deciding. Over time, this work broadened to consider how neural circuits underlie cognition itself.

Zador's plunge into molecular biology began about 6 years ago, thanks, he says, to the support of faculty member Joshua Dubnau, who conveyed some of his expertise during their daily runs in the backroads behind the Lab. For an hour a day, four times a week, Dubnau served as an "advisor, sounding board and tutor."

More recently, working with talented graduate students and postdocs, including Hassana Oyibo, Ian Peikon, and Justus Kebschull, among others, Zador developed his novel neuron-mapping method, dubbed MAPseq (Multiplexed Analysis of Projections by Sequencing).

Visualizing the projectome

Early work with MAPseq set the stage for a broader effort to map the entire "projectome," Zador's first draft of the widely popularized concept of the "connectome." The latter—a wiring map of the brain—has animated neuroscience researchers and helped to inspire President Obama's "Brain Initiative," announced in 2013.



This high-resolution image of neurons and their projections shows the obvious limitations of optical technology in tracing individual neuronal paths.

While the object of a connectome is to map every connection in the brain, the projectome is a major step toward it. "In a limited number of inexpensive experiments, we are already able to indicate the destinations of axons from a vast numbers of neurons. We've focused first on neurons in the cerebral cortex," Zador says.

These data—from the cortex and a few related subcortical regions—have been processed by his team in the form of

a digital 3D rendering of the brain that can be oriented by dragging a computer mouse. These detailed maps are based on data obtained in just a few experiments, performed over several weeks, at a cost of a few thousand dollars.

Moving from a projectome to a connectome—in which not only the destinations but also the connections between nerve-cell projections will be registered—will not be easy, but it is a goal Zador defends as worthy. To date, scientists have generated a full connectome for just one living creature, the tiny roundworm *C. elegans*, with a mere 302 neurons connected by 7,000 synapses. Knowledge of this connectome has not had a transformative impact. But this is precisely because of its simplicity, Zador argues. "When you scale up to a brain with 100 billion neurons, there must be systematic rules and principles that generate the wiring, and knowing these rules is going to be extremely valuable."

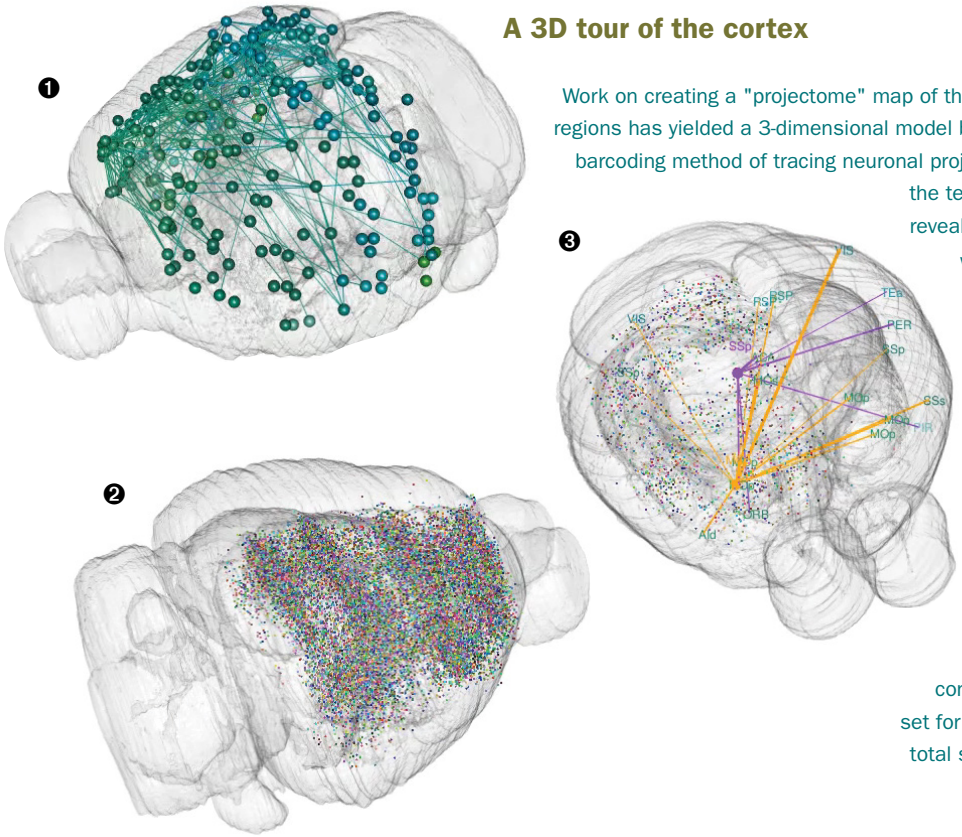
Hence his goal of revealing the mouse connectome, a project with likely implications for numerous human health challenges. "We think important neuropsychiatric disorders like autism and schizophrenia and conditions like addiction derive in part from mis-wiring the brain," he says.

Once researchers have fuller grasp on the range of natural variation in the connectome for healthy mice, they may find specific, characteristic differences in those of mice that model a variety of neuropsychiatric conditions, ranging from autism to schizophrenia to bipolar disorder. At some point down the road, this could lead to therapeutic targets or novel interventions.

More broadly, Zador expects that building a connectome will lead to a specific understanding of how the brain works. He likens attaining this architectural and structural awareness to the ground-breaking discovery of the double-helical shape of DNA. "How does the genetic material replicate? The *structure* of DNA provided the answer to that fundamental question." So, too, Zador hopes, will the structure of the mammalian brain suggest answers to questions about how we go from the material in our heads to the thoughts and inspirations in our minds.

Daniel Dunaief

A 3D tour of the cortex



Work on creating a "projectome" map of the cerebral cortex and several important subcortical regions has yielded a 3-dimensional model based on a single set of experiments using Zador's barcoding method of tracing neuronal projections. These images are stills from an animation the team made, taking viewers on a tour of the cortex as revealed by the approximately 50,000 individual neurons whose axonal paths were traced. In image ❶, each of the green spheres represents a site in the cortex where barcodes were extracted and sequenced. Each contains ~500 to 1000 neurons. The green lines between the spheres represent the strongest connections emanating from each of the spheres. In image ❷, the cloud of multicolored dots—each a barcoded neuron—reminds us that there are actually ~50,000 neurons represented in the green spheres shown in image ❶. In image ❸, the team selected 2 out of the 50,000 neurons and rendered the path of each cell's strongest connections. This is merely a demonstration; the data set for these experiments makes it possible to render the total set of connections for each of the 50,000 neurons.

Challenges that inspire

Many students who start out as STEM majors in college end up leaving STEM. They find the coursework too intense. But students who have presented their research at the DNA Learning Center's Urban Barcode Project and Barcode Long Island symposia gain an early appreciation of how challenging science can be.

High school students chose their own scientific question to explore biodiversity using a technique called DNA barcoding. Much like items in a store are labeled with a unique pattern of lines that make up a printed barcode, organisms have unique patterns in their DNA that can be a genetic "barcode." By comparing the DNA barcodes found in their samples with already-defined DNA barcodes available in online databases, students can identify organisms they study.

Some students are challenged by the sophisticated molecular biology techniques. Others need to overcome their fear of handling the insects they study! But after 5 years and a thousand student barcoders, the DNA Learning Center finds that these challenges foster optimism in participants about a career in science!

One 10th grader offered this advice to future barcoders: "They should learn that even though something doesn't work out the first time they should not be so discouraged and keep trying. You should really be looking to learn from this, not just to succeed."

For more information visit urbanbarcodeproject.org and barcodeli.org

Andrea Alfano

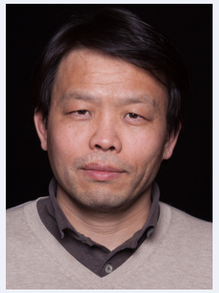
Faculty & Friends

CSHL ♥ New York

The Center for Therapeutics Research (CTR) project is a new $75 million initiative to apply CSHL's biomedical expertise toward advancing therapeutics for genetic diseases. New York State is contributing $25 million for construction and equipment funds to modernize the existing Demerec Laboratory, which was built in 1953 and has housed the laboratories of four of the Lab's eight Nobel Prize winners. "Thank you to all of our friends and supporters on Long Island who made the New York State funding for this project a reality," said President Stillman. "We owe much to the leadership of Senate Majority Leader John J. Flanagan, and our New York State Senator Carl Marcellino. And we are proud to work with our peer research institutions to champion the critical role of the biomedical sector in the Long Island economy."

CSHL leads two international teams

Two CSHL investigators are among 25 international teams that have won 2016 Human Frontier Science Program (HFSP) Research Program Grants: Associate Professor Bo Li, a neuroscientist, and Assistant Professor Je Lee, a genomics expert and cancer researcher. The HFSP grant program supports innovative, cutting-edge research that is expected to open new fields of investigation. Dr. Li's project is "Single cell resolution imaging and optogenetics in the amygdala fear circuits in behaving animals." Joining Li in this collaborative research are teams from University Paris Descartes, CNRS, France; MIT; and The Hebrew University, Israel. Lee's project is: "Complete cell lineage trees inferred by *in situ* genotyping of induced somatic mutations." Joining in his research are teams from Institut de Génomique Fonctionelle de Lyon, France; and University College, London.



Pershing Square Sohn Prize for next-gen cancer therapies

Associate Professor Chris Vakoc, M.D., Ph.D., was awarded the third annual Pershing Square Sohn Prize for Young Investigators in Cancer Research to help fund his explorative and high-risk/high-reward research. A finalist in last year's prize, Vakoc's research employs a novel CRISPR technique that can reveal individual protein domains that sustain cancer cells. His lab is now deploying this technology in a diverse array of human cancers to reveal therapeutic opportunities and basic mechanisms of cancer gene control. "The Pershing Square Sohn Prize Winners are among the most talented and innovative scientists in cancer research," said Olivia Tournay Flatto, President of the Pershing Square Foundation. "Dr. Vakoc's research has great promise for guiding the development of next-generation cancer therapies."

Plant biology program sees the light

Welcome Ullas Pedmale, Assistant Professor, from a post-doctoral fellowship at the Salk Institute of Biological Sciences. With a Ph.D. from the University of Missouri-Columbia, he has been exploring changes in plant architecture in response to variations in light quality. Plants don't have specific organs that see or hear, but they modify their development according to external signals. At CSHL, his lab studies how the environment of a plant modulates its development. "Understanding environmental control of growth will have far-reaching implications for agriculture, energy production, and other human activities," Pedmale explains.

Joining a legacy of young leaders

Assistant Professor Camila dos Santos is a 2016 Rita Allen Foundation Scholar, an award that recognizes young leaders in biomedical science whose research holds great promise for revealing new pathways to advance human health. Camila joins a distinguished list of CSHL faculty whose early career research was also supported by the foundation, including Bruce Stillman, David Tuveson, Doug Fearon and Greg Hannon. In recent years, CSHL winners have included: Lloyd Trotman, Chris Hammell, and Molly Hammell. Dos Santos' lab is studying breast development in mouse models and has found dramatic differences between first and second pregnancies, which appear to be mediated by epigenetic mechanisms—molecular changes that affect gene expression without altering DNA sequences. This award will allow the dos Santos lab to assess the relevance of these epigenetic phenomena for breast cancer risk, which is at least 30 percent lower in women who have a full-term pregnancy before the age of 25. A long-term goal is to "propose a prophylactic strategy" so that even women who do not have an early pregnancy can "still leverage the preventive effect that the pregnancy signals are bringing to decrease the risk of breast cancer," dos Santos says.

RITA ALLEN FOUNDATION

Women's Partnership lauds CSHL alumna

"Evelyn Witkin was a graduate student here in 1944, and last year won the Lasker Prize, which is the most prestigious medical research prize in the United States, for work that she started at Cold Spring Harbor Laboratory all those years ago," explained CSHL President Bruce Stillman as he introduced her as this year's Women's Partnership for Science lecturer. "I really can't overstate the great good fortune I had of spending those years at Cold Spring Harbor, and being witness to, and in a small way a participant in, the birth of molecular biology," said Witkin. Since 2002, the event has raised $1.7 million. Thanks to this year's co-chairs: E. Ainslie, L. Bahnik, M. Celestino, S. Cohen, K. Perkin Davison, T. A. Dellomo, C. Gero, A. Lamb, A. Lister, J. Mercer, M. Nagel, J. C. Nicholls, L. Parent, P. Petersen, L. Piazza, W. Posillico, Dr. M. Simons, H. Geier Smith, C. Stebbins, S. Tytel, and M. van de Stouwe, M.D. Joining Witkin in this photo are CSHL postdoctoral fellows Olivia Mendivil Ramos, Sarah Diermeier, and graduate student Brittany Cazakoff.

New on Board

The CSHL Board of Trustees elected Douglas Schloss as its newest member. Since 1994, Mr. Schloss has been CEO & Managing Member of Rexford Management. He previously managed arbitrage and investment activities at Marcus Schloss & Co. Mr. Schloss is a graduate of Princeton University and Harvard Business School. A 1977 graduate of St. Paul's School, he served as the President of the Board of Trustees of the college preparatory school from 2004 to 2014. "We welcome Doug's unique combination of business expertise and leadership experience in the non-profit education sector," said CSHL Chairman Jamie C. Nicholls.

A night at the Museum

The 11th Double Helix Medals celebrated Alan Alda and Dr. P. Roy Vagelos at the American Museum of Natural History, raising $4+ million for the Lab's research. Legendary actor and science communicator Alda was lauded for inspiring public understanding of science. Biopharmaceutical visionary Vagelos was honored for philanthropy in human health, biomedical research and education. Visit cshl.edu/DHMD for more.

CAC2 PEDIATRIC CANCER RESEARCH CONFERENCE 2016

Childhood cancer community unites at the Lab

Together with the Coalition Against Childhood Cancer (CAC2), CSHL hosted the first ever "From Bench to Bedside and Beyond" conference convening a wide range of childhood cancer community stakeholders at CSHL to advance research. Bruce Stillman opened the 2-day program, followed by presentations from CSHL Associate Professors Chris Vakoc and Mickey Atwal, speakers from the National Cancer Institute, the nation's top cancer basic and comprehensive cancer centers, and the pharmaceutical industry. They addressed the latest technologies, the clinical pipeline, clinical trials and today's more promising therapeutics. CSHL proudly joined this effort also supported by sponsors Alex's Lemonade Stand Foundation, Bristol-Myers Squibb, United Therapeutics and Amgen. Serving a bridging role for childhood cancer stakeholders across the nation, CAC2 has over 90 member organizations and more than 50 individual and student members.

A joint appointment at CSHL and Northwell

"We are pleased to announce that Robert D. Maki, M.D., Ph.D., will lead the joint CSHL-Northwell Health Cancer Institute initiative to establish a Cancer Clinical Research Center that will translate the outstanding basic science at CSHL into the clinic, said Bruce Stillman, President of CSHL. "He is an internationally renowned sarcoma cancer physician and researcher who will work closely with oncologists at Northwell and scientists at CSHL to advance cancer treatment." Maki is a CSHL professor, member of CSHL's NCI-designated Cancer Center and the director of the Center for New Cancer Therapies at the Northwell Health Cancer Center. He will play a key role in the strategic affiliation between Northwell and CSHL, established in 2015 to accelerate cancer research, diagnosis and treatment. Maki's position as director includes building a portfolio of clinical research and exploring unique therapeutics for all cancers, which will increase cutting-edge treatment options for patients throughout the healthcare system.

Join our Drive for 125

Impact the future with a legacy gift to Cold Spring Harbor Laboratory

"I was fortunate to be a Ph.D. student at CSHL in Bruce Stillman's lab, from 1984–1988. What an adventure it was! Imagine the thrill of a naïve but eager student being thrust into top-level science, trying to discover something that no one has ever known before. I'm proud to be part of the CSHL family.

Why did I decide to contribute financially to CSHL's future? It's quite simple. The lab is a scientific treasure. It's a place where research continues to be done at the highest level, it is a training ground for young scientists, it is THE meeting place for scientific conferences, and it preserves the history of molecular biology. We need more Cold Spring Harbor Laboratories in the world."

~Greg Prelich, Ph.D.

To discuss making a gift to CSHL, contact Diane Fagiola at 516-367-8471 or email fagiola@cshl.edu

Greg, a former CSHL student, is a new member of the Helix Society.

CSHL Association

CSHL Association comprises some 1000 neighbors and friends of the Laboratory who contribute to the Annual Fund, an essential source of unrestricted support for outstanding young scientists. Association members get to know CSHL scientists at lectures, concerts, dinners and other social events that support the Laboratory. Membership levels start at $100 per year. For more information please contact Karen Orzel, Director, Annual Giving and Donor Relations, at 516.367.6886 or orzel@cshl.edu.

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Co-hosted by a pair of science enthusiasts, each episode tells a colorful story, bringing genetic research to life:

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3. Non-modern Family
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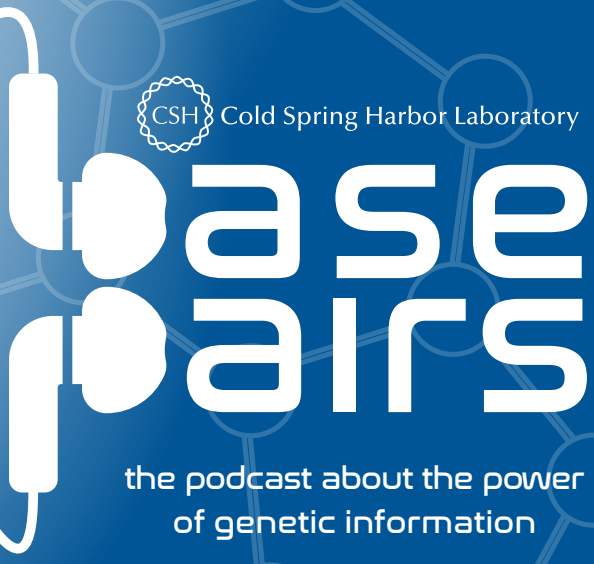
6. When Antioxidants are Pro-cancer
7. The Brain Atlas



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