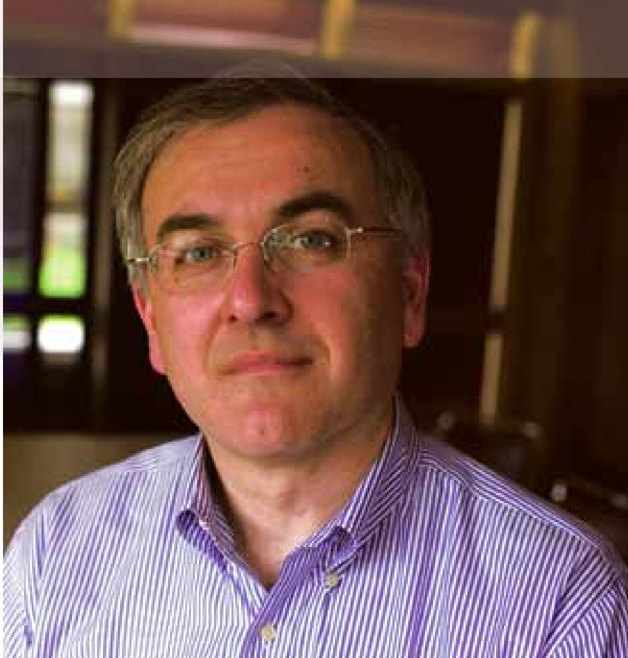


RESEARCH PROFILE

Adrian Krainer

Tinkering with the cell's RNA-editing process yields payoff in treating a deadly genetic disease

Tucked into a list of several dozen neuromuscular disorders is a killer of a disease called Spinal Muscular Atrophy (SMA). Although not as well known as Lou Gehrig's, Duchenne's, or other muscular dystrophies, SMA is, in fact, the No. 1 genetic cause of death among children under the age of two. At Cold Spring Harbor Laboratory, Adrian Krainer is working steadily to wipe SMA off this list.

Spurring him toward success are his breakthroughs over the last 25 years in deciphering the intricacies of alternative splicing, a cellular process for editing RNA — the chemical cousin of DNA. In the last five years, Krainer has used his insights into this process to correct the splicing defect that causes SMA, in systems of increasing complexity — first in test tubes, then in cells taken from SMA patients and grown in the lab, and most recently, in genetically engineered mouse models of SMA.

"A few more key steps remain before we can petition the Federal Drug Administration for clinical trials," cautions Krainer. Still, his SMA-focused efforts have the potential for a wider payoff. His strategy, honed on the genetics of SMA, could be applicable to other human diseases caused by defective splicing.

An early, cell-free success

When a gene is switched "on" or expressed, its DNA is decoded letter-by-letter into RNA in several steps. The first RNA draft—called pre-messenger RNA (pre-mRNA)—includes chunks of useless information, called introns. So like extra footage cut out of a film, these bits of RNA are lopped off and the remaining bits, called exons, are spliced together to make messenger RNA, which then gets translated into a protein.

Rich Roberts, then at CSHL, and Phil Sharp of MIT — who both later won the Nobel Prize in 1993— discovered RNA splicing in 1977, the year Krainer arrived from his native Uruguay to enroll as an undergraduate at Columbia University. When he began graduate studies at Harvard University four years later, scientists were still unclear about how splicing worked and what molecules were involved.

"It was difficult to get at this question because of problems with reproducing this process in a test tube," recalls Krainer, who soon accomplished this feat. With components extracted from cells that had been split open, he developed an efficient "cell-free" system that is still used by scientists to work out the rules and steps of splicing. Krainer and his colleagues proved the system's usefulness right away by recreating disease-causing splicing defects in the test tube.

Presenting this work at a CSHL meeting in 1984 and at an international meeting in Rome in 1985, he caught the eye of CSHL's Roberts, who was trying to recruit promising young talent. For Krainer, the road from Rome led straight to CSHL. He arrived on campus in 1986 as the first member of the CSH Fellows program, in which newly minted Ph.D.s and M.D.s tackle independent research projects before taking faculty positions.

Switching to alternative splicing

Since that time, Krainer has discovered many of splicing's principles, unraveled how splice-altering mutations can cause disease, and concocted ways to correct faulty splicing. His research group also explores how numerous proteins and RNAs involved in splicing can multitask and regulate a host of other essential processes inside the cell. Krainer was the first, in fact, to discover one of these splicing proteins, in 1989, after a long hunt. For three years, he and a technician grew "liters of cells" and

fractionated their extracts for countless hours in room-sized refrigerators before finding the elusive prize, which now goes by the name SF2/ASF. Today, more than 200 splicing proteins have been found. "Had I known then how complicated the picture of splicing would get, I might not have started down this road," he jokes ruefully.

The cell's splicing machinery includes more than just enzymes that cut out introns and paste together exons. SF2/ASF, for example, promotes splicing by acting as a bridge: one end sticks to the pre-mRNA, while the other end tethers protein and RNA components that eventually do the actual cutting and pasting.

While exploring the activity of splicing factors, Krainer, who was appointed full professor in 1994, came to appreciate the power of alternative splicing, which allows a single gene to give rise to multiple versions of a protein. Via this process, a gene's exons can be mixed in different combinations to generate different messenger RNAs, each carrying the recipe for a different protein.

Krainer's team found that the choice of which exons to include is guided by splicing proteins like SF2/ASF, which stick to pre-mRNA at specific sites near the exons called "enhancers." This guiding is dosage-dependent — different protein isoforms are made depending on how much SF2/ASF is available. Working with bioinformatics experts at CSHL, including Michael Zhang's group, Krainer's team has developed computational tools to identify splicing enhancers and their counterparts—called splicing silencers—within exon and intron sequences of large genomes, including that of humans.

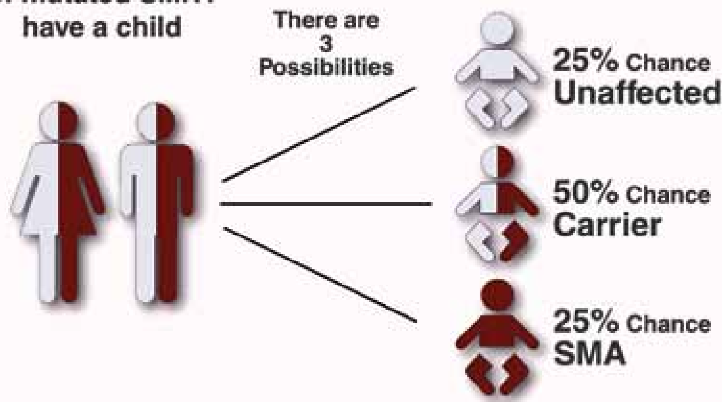
A few years ago, Krainer began focusing on splicing defects caused by mutations, many of which had been linked to catastrophic diseases, including a host of neurological disorders. "Such mutations cause exons to be unintentionally included or crucial exons to be erroneously skipped, leading to missing or poorly functioning proteins," he explains.

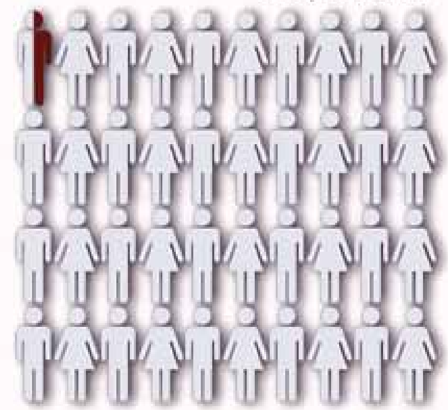
Fixing SMA

A splicing-related neuromuscular disease that grabbed his attention was SMA, which is caused by a deficiency of the SMN (Survival of Motor Neuron) protein. When SMN levels are low in the spinal cord's motor neurons, they and the muscles they control waste away. Hence babies born with SMA progressively lose the ability to move, swallow, and breathe.

SMN protein is produced by the *SMN1* gene, which is deleted or mutated in SMA patients. Humans have a second gene, *SMN2*, which produces an identical protein. But *SMN2* is a poor backup. It differs from the *SMN1* gene by a single DNA "letter"—a T (thymine) instead of C (cytosine)—near the start of exon 7. This minuscule change causes this exon to often be skipped during splicing, resulting in low levels of full-length SMN protein.

Bench work: Adrian Krainer discusses splicing experiments with postdoctoral researcher Mads Jensen (left photo) and Research Investigator Yimin Hua (right photo)





Krainer and former postdoc Luca Cartegni found that this skipping occurred at least in part because SF2/ASF failed to attach to the correct enhancer element in the SMN2 pre-mRNA. To correct the error, they created a "designer" molecule – a synthetic chimera in which SF2/ASF's enzyme-binding part was joined to a custom-designed synthetic RNA that can be made to bind any sequence within cellular pre-mRNA by pairing up with its RNA "letters."

So even if a cell's own SF2/ASF performs faultily, its synthetic replacement gets the job done. Krainer's team has proved this strategy's versatility by using it to correct a splicing defect in *BRCA1*, the breast cancer gene. His lab continues to explore the connections between defective splicing and cancer.

The SMA team, led by Research Investigator Yimin Hua, is currently moving forward with another, simpler, synthetic molecule called an anti-sense oligonucleotide (ASO), which works much more efficiently in cells. The ASOs, developed in collaboration with California-based Isis Pharmaceuticals (and Massachusetts-based Genzyme Corporation), can fix SMN2 splicing in test tubes, in patients' cells grown in the lab, and in mice that have been engineered to carry a human *SMN2* gene. "The ASO, delivered straight into the fluid that surrounds the brain and spinal cord, protects nerve cells, improves muscle function, and prolongs the animals' lives," explains Krainer.

In a parallel approach with Boston-based Paratek Pharmaceuticals and former postdoc Michelle Hastings, now at Rosalind Franklin University in Chicago, the team has found a molecule that resembles the common antibiotic tetracycline, which also boosts SMN levels in mouse models. This collaboration, initiated by funding from the patient-support group Families of SMA, is now focusing on preclinical drug development.

As Krainer marches on toward translating these successes into viable clinical therapies, he is well aware of the challenges that remain. So are the many parents of SMA-affected kids, who attend his presentations at SMA support group meetings.

"The parents ask some of the most insightful questions about the data that I present," says Krainer. "When we start discussing the nitty-gritty of various splicing mechanisms, I try to convince them to drop what they're doing and come join my research team."

"In some ways, we've moved faster than I thought we could when I first started working on SMA 10 years ago. But in other ways, it never moves fast enough."

Hema Bashyam

Fighting SMA

Krainer is an active participant in organizations like Fight SMA and Families of SMA, and also interacts with the SMA Foundation, and the Muscular Dystrophy Association, all of which have raised funds to support several of his research projects over the years. He often accompanies SMA-affected families to speak with legislators to advocate for the SMA Treatment Acceleration Act, which aims to increase federal funding for SMA research and coordination between clinical centers.

"SMA research is providing valuable insight into so many splicing-related genetic diseases," explains Krainer. Sharing this opinion, the National Institutes of Health chose SMA to be a model for translational research, fast-tracking efforts to bring lab-based discoveries to the clinic.