Highlights of the Year

Research

In 2023, hundreds of scientists working in more than 60 Cold Spring Harbor Laboratory (CSHL) research groups published their findings in the world's major scientific journals. Their efforts reflect the full spectrum of CSHL's programs in Cancer, Neuroscience, Plant Biology, Quantitative Biology, and Genomics. The following is a sampling of this year's important findings.

A Secret Weapon Exposed

RNAs are having a moment. The foundation of COVID-19 vaccines are messenger RNAs (mRNAs) that encode protein antigens. They have made their way from biochemistry textbooks into popular discourse. There are many types of RNA in our cells. Unfortunately, when some of them malfunction, it can result in cancer or developmental disorders. Our cells have molecular "machines" that eliminate RNAs at the right time and place. Most come equipped with a "motor" to generate the energy needed to untangle RNA molecules. But one machine, Dis3L2, is an exception. The enzyme can unwind and destroy RNA on its own. This has puzzled scientists for years. Finally, CSHL biochemists have pieced together what is happening.

L. Joshua-Tor

Using state-of-the-art molecular imaging technology, CSHL Professor and HHMI Investigator Leemor Joshua-Tor captured Dis3L2 at work. She fed the molecular machine hairpin snippets of RNA and imaged them getting "eaten" at various stages. After the machine had chewed up the tip of the RNA, it swung open a big arm of its body to peel apart the hairpin and finish the job. Joshua-Tor's team discovered that Dis3L2 has a protruding wedge that enables it to unwind RNA. If the researchers removed the wedge, Dis3L2 could no longer untangle the RNA hairpin.

The findings reveal a surprising new way in which our cells' RNA-controlling machines execute their tasks. Rather than solid structures, these molecular workhorses need to be considered malleable and versatile. "We have to start thinking about these proteins as much more dynamic entities," Joshua-Tor says, "and take that into account when we design therapeutics." This new outlook may help scientists develop better treatments for diseases and disorders caused by RNA gone haywire.

Shape-Shifting Antibiotics

J.E. Moses

In the United States alone, drug-resistant bacteria and fungi infect almost three million people per year and kill about 35,000. Now, CSHL Professor John E. Moses has created a new weapon against these drug-resistant superbugs—an antibiotic that can shape-shift by rearranging its atoms.

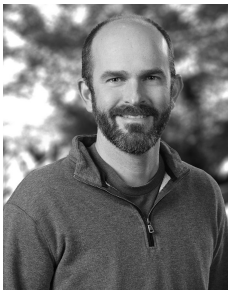
Moses came up with the idea of shape-shifting antibiotics while observing tanks with rotating turrets in military training exercises. A few years later, Moses learned of a molecule called bullvalene, whose atoms can swap positions. This gives it a changing shape with over a million possible configurations—exactly the fluidity Moses was after.

Several bacteria have developed resistance to a potent antibiotic called vancomycin, used to treat everything from skin infections to meningitis. Moses thought he could improve the drug's performance by combining it with bullvalene. He turned to click chemistry, a Nobel Prize–winning class of fast, high-yielding chemical reactions that "click" molecules together reliably. Using this technique, Moses and his colleagues created a new antibiotic with two vancomycin "warheads" and a fluctuating bullvalene center.

They found the shape-shifting antibiotic significantly more effective than vancomycin in treating a deadly superbug called vancomycin-resistant *Enterococci* (VRE). Remarkably, the bacteria did not develop resistance to the new antibiotic.

Once Sarcoma, Now Muscle

"Every successful medicine has its origin story. And research like this is the soil from which new drugs are born," says CSHL Professor Christopher Vakoc.

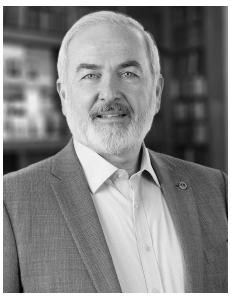


C. Vakoc

A devastating and aggressive type of pediatric cancer, rhabdomyosarcoma (RMS), resembles children's muscle cells. For six years, Vakoc's laboratory has been on a mission to transform sarcomas into regularly functioning tissue cells through a method known as differentiation therapy. To carry out their mission, Vakoc's team created a new genetic screening technique. Using genome-editing technology, they scanned for genes that, when disrupted, would force RMS cells to become muscle cells. That is when a protein called NF-Y emerged.

With NF-Y impaired, the scientists witnessed an astonishing transformation. "The cells literally turn into muscle," Vakoc recalls. "They're switching from a cell that just wants to make more of itself to cells devoted to contraction." The newfound relationship between NF-Y and RMS may set off the chain reaction needed to bring differentiation therapy to patients. The mission does not stop at RMS. Differentiation therapy could be applicable to other cancer types. For example, Vakoc and his team have already succeeded in transforming Ewing sarcoma cells into healthy tissue cells. Notably, both the Ewing sarcoma and RMS discoveries were supported by local families who had lost loved ones to these cancers. Those families and Vakoc's laboratory may yet become the heroes of a new origin story—a scientific breakthrough that could someday help save children's lives and revolutionize cancer treatment as we know it.

From Tragedy to Triumph



A. Krainer

CSHL Professor Adrian Krainer is best known for his groundbreaking research on antisense oligonucleotides (ASOs)—molecules that can control cells' protein levels. His efforts led to Spinraza®, the first FDA-approved treatment for spinal muscular atrophy (SMA).

Following his success with SMA, Krainer started looking into other diseases in which ASOs could make a difference. He soon set his sights on a lethal pediatric brain cancer called diffuse intrinsic pontine glioma (DIPG). "I was contacted by a neurologist and his friend, who had lost her child to DIPG," Krainer explains. Now, Krainer's laboratory has found a way to increase survival rates in mice with DIPG using ASO technology similar to that used for the development of Spinraza®.

The new ASO works by shutting down a mutated protein called H3.3K27M. In DIPG, the dominant mutation blocks closely related proteins from turning many genes on and off, leading to uncontrolled cell growth. When the team used the ASO on mice with DIPG, the genes it affected returned to normal. The tumors stopped growing as fast, and the animals lived longer.

"We could see a lot fewer proliferating cells, and the tumor cells were differentiating into healthy nerve cells," Krainer says. "That tells us DIPG's malignant changes are reversible to an extent."

S. Beyaz

Cancer's Multiple Personalities

Cancer is very complex. Mutations in the same genes can lead to different tumor subtypes in different people. Highly similar proteins produced from the same gene are called isoforms. Different isoforms generate different tumors through a process known as exon skipping. Now, CSHL Assistant Professor Semir Beyaz has developed a new method to model certain liver tumor subtypes using the gene-editing tool CRISPR-Cas9.

Beyaz and his colleagues produced two distinct tumor subtypes by targeting a single section of the mouse gene *Ctnnb1* with CRISPR-Cas9. This tool is mostly used to inhibit gene function. In fact, this is the first time CRISPR-Cas9 has been used to generate different cancer-causing gain-of-function mutations in mice. These mutations enhance protein activity to promote tumor growth. Beyaz's team sequenced each tumor subtype to figure out which isoform was associated with the differences they observed. Next, to confirm that these isoforms actually caused the variances, they produced them in the mouse without using CRISPR. They found that they were indeed able to generate the two different tumor subtypes along with their respective characteristics. Both subtypes are also found in humans.

The mutations Beyaz targeted can lead to colon and liver cancers. Targeting exon skipping has emerged as a potential therapeutic approach for treating cancer and other diseases. Beyaz's method allows researchers to investigate this phenomenon in living mouse cells using CRISPR-Cas9 technology. The platform could someday help researchers develop new therapeutic interventions, such as ASOs that can modulate exon skipping.

These Worms Have Rhythm

Growing from a tiny cluster of cells into an adult organism takes precise timing and control. The right genes must turn on at the right time, for the right duration, and in the correct order.

C. Hammell

CSHL Professor Christopher Hammell found that in the *C. elegans* worm, this genetic orchestra has no single conductor. Instead, a quartet of molecules works in concert to time each developmental stage. Each begins with two proteins, NHR-85 and NHR-23. Together, they spark a pulse of gene expression, switching on the microRNA *lin-4*, which controls stem cell development patterns. The pulse's timing, strength, and duration depend on the short period of time when NHR-85 and NHR-23 interact with each other. The pulse of gene expression of the microRNA *lin-4* is terminated by another protein called LIN-42, which ends each developmental period by shutting off NHR-85.

Hammell teamed with Wolfgang Keil from the Curie Institute in Paris, France, to observe this gene expression cycle in action. Their collaboration resulted in the first-ever video footage to capture active gene expression throughout an entire animal in real time. Understanding how the worm's developmental clock is regulated could help explain how time affects development in other animals, including humans.

The Science of Supermoms

When you pick up a baby, you can't help feeling happy. But where does this feeling come from? How does it influence future behavior? CSHL Professor Stephen Shea has found that, when it comes to maternal care, social contact is its own reward.

S. Shea

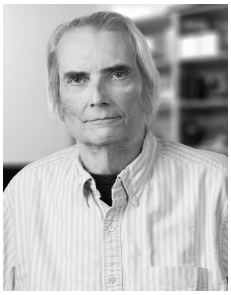
Shea and postdoc Yunyao Xie traced maternal motivation in mice to a region of the brain called the ventral tegmental area (VTA). They found that neurons in the VTA use dopamine to nurture maternal instincts through a process called reinforcement learning. When a mom picks up a crying child, the mother's brain's VTA neurons release dopamine. This creates an expectation of future rewards, driving her to pick the child up again the next time it cries.

To observe reinforcement learning in action, Shea's team built an enclosure with two chambers. They placed a mouse on one side and a pup on the other, behind one of two doors. They then played specific sounds to signal whether the pup was present and if it was behind door number one or two. Each time the mouse heard a sound and retrieved the pup, VTA neurons rewarded it with dopamine.

Deciphering how social contact is encouraged in mice gives researchers a clue about VTA neurons' involvement in rewarding other behaviors. This may lead to a better understanding of neurodevelopmental conditions that affect social interactions, like autism.

Holy Immunity, Bat Genomes!

CSHL Professors W. Richard McCombie and Adam Siepel and postdoc Armin Scheben have sequenced the genomes of the Jamaican fruit bat and Mesoamerican mustached bat. Comparing these sequences to those from other mammals, the team found that rapid evolution has streamlined bat genomes to defend against infection and cancer.



W.R. McCombie

A. Siepel

The Jamaican fruit bat and Mesoamerican mustached bat belong to the world's most ecologically diverse superfamily of mammals. McCombie, Siepel, and Scheben created complete genomes for both bats using new Oxford Nanopore sequencing technology. They then compared these sequences to 15 other bat and mammal genomes, including humans. This analysis revealed an unknown shift in levels of two inflammatory protein–coding genes called interferon-alpha and -omega.

The team also found that compared with other mammals, bat genomes contain more changes in cancer-related genes, including six that repair DNA and 46 that suppress tumors. McCombie, Siepel, and Scheben are currently exploring how bats' immune genes are regulated and how they might be expressed in different parts of the body. They hope their work will provide new insights into the links between immunity, aging, and cancer.

Plants Pass on Memories

CSHL Professors and HHMI Investigators Rob Martienssen and Leemor Joshua-Tor have been researching how plants pass along the markers that keep transposons inactive. To silence them and protect the genome, cells add regulatory marks to specific DNA sites. Martienssen and Joshua-Tor have now shown how a protein called DDM1 makes way for the enzyme that places these marks on new DNA strands. Plant cells need DDM1 because their DNA is tightly packaged. To keep their genomes compact and orderly, cells wrap their DNA around proteins called histones.

R. Martienssen

Martienssen and former CSHL colleague Eric Richards first discovered DDM1 30 years ago. Now, through genetic and biochemical experiments, Martienssen has pinpointed the exact histones DDM1 displaces. Joshua-Tor used cryo-electron microscopy to capture detailed images of the enzyme in action. Together, they saw how DDM1 grabs onto particular histones to remodel packaged DNA. "An unexpected bond that ties DDM1 together turned out to correspond to the first mutation found all those years ago," Joshua-Tor says.

The experiments also revealed how DDM1's affinity for certain histones preserves regulatory controls across generations. The team showed that a histone found only in pollen is resistant to DDM1 and acts as a placeholder during cell division. "It remembers where the histone was during plant development and retains that memory into the next generation," Martienssen says.

Plants may not be alone here. Humans also depend on DDM1-like proteins. The new discovery may help explain how those proteins keep our genomes functional and intact.

Autism in the Family Tree

Scientists long thought that siblings born with autism spectrum disorder (ASD) share more of their mother's genome than their father's. But CSHL Professors Ivan Iossifov and Michael Wigler have now shown that, in many cases, it is dad who might be playing a bigger genetic role.

Over the last two decades, CSHL scientists have led a multimillion-dollar effort to uncover the genetic origins of autism. They discovered thousands of genes that, when damaged, may cause a child to be born with ASD. However, their work was not able to account for all cases of ASD. So, Iossifov and Wigler set out to find the missing sources.