In 2021, hundreds of scientists working in nearly 60 Cold Spring Harbor Laboratory (CSHL) research groups published their findings in the world’s major research journals. Their efforts reflect the full spectrum of CSHL’s programs in Cancer, Neuroscience, Plant Biology, Quantitative Biology, Genomics, and a new chemistry lab. The following is a sampling of this year’s important findings.

Pinpointing Cancer’s Origins
CSHL Adjunct Professor Pavel Osten and Professor Lloyd Trotman have pioneered a new method to track how prostate cancer progresses in mice, from its birth to its spread into other tissues. The pair combined their expertise in whole-organ imaging and prostate cancer to track how a single cancer cell can grow into a tumor and spread to other organs. For the first time, researchers can study a tumor as it grows in a setting that accurately mimics the disease in real life. The study led by Julian Taranda, a former postdoc in the Osten lab, was published in *Cell Reports*.

The researchers used a virus to transform as little as one normal mouse prostate cell into a cancerous cell. This lone cancer cell was located using a microscope technique called whole-organ serial two-photon tomography. The tomography machine is fully automated. It takes an image of all the cells on the top layer of an organ, slices off that piece, images what is in the next layer, and repeats the process until it has photographed the entire organ. Then, using artificial intelligence, a computer creates a 3D reconstruction of the organ at single-cell resolution. The scientists hope this versatile new method will help tackle unexplored questions about the early steps of cancer’s growth and escape into other organs, wherever it starts.

Cushy Homes for Cancer
Cancer cells live in a complex neighborhood populated by immune cells, blood vessels, and other structures. Cells signal each other who they are and what they want. Cancer cells may hide their identity so they can grow and spread more easily. CSHL Professor Mikala Egeblad wants to know what creates an ideal microenvironment for a wandering cancer cell. She reasons that if she can decode the signals in the neighborhood, she may find ways to harness those signals to defeat cancers.

Breast and ovarian cancer cells can reprogram immune cells to make them into “tumor-associated macrophages” (TAMs), which act like a security team hired by the cancer cells to protect themselves. Egeblad’s lab found that interferon-gamma plus an immune system activator re-reprogrammed breast cancer TAMs so they would attack the cancer. The two drugs were also extremely effective against ovarian cancer in mice. In both cancers, the treatment slowed metastases and made ovarian tumors more susceptible to chemotherapy.

Lessons learned about the cancer microenvironment can be applied to COVID-19. Neutrophils, a type of immune cell, can form sticky neutrophil extracellular traps, or NETs, which normally trap pathogens. However, too many NETs can be toxic, especially in the lungs, where they can damage tissue and cause respiratory distress. Thus neutrophils, like macrophages, could become therapeutic targets.

Cancer’s Sweet Tooth
CSHL Professor Christopher Vakoc and his lab discovered that acute myeloid leukemia (AML) cells depend on a single transporter to get the essential sugar inositol into the cell. Cancers streamline certain cell processes, “putting all their eggs in one basket” with a single pathway. Vakoc can then develop treatments to knock out that remaining pathway and kill the cancer cells.
Most cells either get inositol from the bloodstream (it is present in many foods) or make it themselves. Since there is plenty of sugar available outside the cell, some cancer cells decided to rely on the inositol transporter to capture it and stop making it inside the cell. If researchers can find a treatment that can turn off or block this transporter, the cancer cells would starve. This method would leave normal cells unharmed since they can make inositol on their own. Vakoc reported his findings in Cancer Discovery.

Vakoc says his work suggests a few roads to developing a therapeutic: “You could make an antibody that just sticks to this transporter. It doesn’t need to get into the cell, and it could shut off the transport function. The other possibility, from a drug development point of view, is inositol. You could build a molecular medicine that sort of looks like inositol, but has a few chemical differences that can clog the transport function.”

Predicting Cancer’s Path
Assistant Professor David McCandlish and collaborators used the statistical method of density estimation in a new way: to predict how combinations of genetic mutations cause different types of tumors. McCandlish says, “This is what’s fascinating about mathematical research. Sometimes you see connections between topics that seem so different, but at a mathematical level, they use the same ideas.”

McCandlish mapped the combinations of mutations most likely to occur in a particular protein and in the same cancer cell. It is straightforward to predict the co-occurrence of a couple of events, like how often you might find two people of the same height in a group.

But for complex biological sequences, such as the hundreds of amino acids that make up a protein, predicting the probability of each potential sequence becomes astonishingly complex. “Sometimes, with one mutation in a protein sequence, the protein works fine,” explains McCandlish. “And with a second mutation, it still works fine, but then if you put the two of them together, you get a broken protein. We’ve been trying to come up with methods to model interactions between any number of mutations.” Their new method can predict how hundreds of thousands of different mutation combinations impact the function of a protein.

The team published the study in the Proceedings of the National Academy of Sciences and made their density estimation software available publicly.

Estrogen Gives Mice the Moves
Female animals are most active when estrogen levels are high, increasing their chances of encountering a mate when pregnancy is most likely. Mice with low levels of estrogen are more sedentary than ones with high levels. Women also become more sedentary as estrogen levels decrease during menopause. CSHL Assistant Professor Jessica Tollkuhn and her collaborators at the University of California, San Francisco, have now traced this hormone-driven activity to a cluster of estrogen-sensitive cells in the brain.

Tollkuhn and her team study the profound impact estrogen has on the brain, where it not only influences activity levels, but also modulates mood, alters sleep patterns, and helps control body temperature. In the brain, the hormone latches onto an estrogen receptor.
(ER-alpha), changing the activities of specific genes. Tollkuhn and colleagues have found nearly 2,000 sites within the genome that interact with ER-alpha, suggesting the hormone regulates hundreds of different genes in the brain.

One of those genes is Mc4r. The team’s experiments in mice revealed how the hormone provokes signaling changes inside estrogen-sensitive neurons. Importantly, they found they can mimic these effects without increasing estrogen exposure, simply by activating Mc4r in the relevant neurons.

The team’s findings suggest it may be possible to develop targeted therapies that restore specific benefits of estrogen signaling, without the side effects of hormone replacement.

**Every Brain Cell Counts**

CSHL Adjunct Professor Pavel Osten and his lab mapped cells and connections within the mouse primary motor cortex. They categorized different cell types throughout the brain in a quantitative brain-wide (qBrain) catalog. With this method, the researchers can standardize a 3D map of the 100 million neurons in the mouse brain and in the near future, even the 100 billion neurons in the human brain.

Osten’s technique starts with labeling brain cells of interest to identify classes of cells or particular pathways. The brain is then preserved and imaged automatically at high resolution. Each brain is analyzed by a computer, which can count the cells or trace the pathways, comparing it to previously mapped brains. The entire process, automated after the brain is preserved, takes 12 to 32 hours per mouse. The researchers then compare new brains to their standardized 3D maps to figure out gender differences within a species, development stages, and diseases. They already discovered anatomical differences between male and female mouse brains associated with behavioral differences.

Osten and his lab were a part of the founding group of scientists for the NIH-funded Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative – Cell Census Network (BICCN). Similar in scope to the Human Genome Project, BRAIN is providing a foundation for studying the mammalian brain with new methods.

**Helpless No More**

Everyone faces stress occasionally, whether in school, at work, or during a global pandemic. CSHL Professor Linda Van Aelst studies how individuals respond to stress. Her lab studied the mouse gene Ophn1, which plays a critical role in developing brain cell connections, memories, and stress tolerance. When Ophn1 is removed in a specific part of the brain, mice express depression-like helpless behaviors. The researchers found three agents, including a drug called Fasudil, that could reverse this effect.

To test for stress responses, the researchers put mice into a two-room cage with a door in between. The floor in one room provides a light shock to their feet, and normal mice leave that room. But animals lacking Ophn1 sit helplessly in that room without trying to escape.
Her lab deleted the *Ophn1* gene in different brain regions. Removing *Ophn1* from a circuit in the prefrontal cortex known to influence behavioral responses and emotion induced the helpless phenotype. Overactivity in a particular part of the circuit was the key.

In humans, mutations in the *Ophn1* gene cause a rare X-linked disease that includes poor stress tolerance. Van Aelst hopes that understanding the complex circuit behind *Ophn1*-related stress responses will lead to better treatments for humans. The team published their results in *Neuron*.

### Robots Explain Their Thoughts

Brain-like artificial networks are often referred to as a “black box” because researchers do not know how they learn and make predictions. CSHL Assistant Professor Peter Koo and his team reported new ways to peek inside the box and identify key features on which the computer program relies, particularly when trying to analyze complex RNA and DNA sequences.

Residual Bind is a type of AI program called a deep neural network. It predicts the ability of RNA sequences to bind to proteins. Koo and his team developed a new method, called global importance analysis, that “quizzes” this AI program to figure out what rules it learned on its own and if they are the right ones. They discovered the network considered more than just the spelling of a short stretch of RNA. It factored in how the RNA strand might fold over and bind to itself, how close one RNA pattern is to another, and other features.

Koo’s team also reported a new way to train a type of AI network, called a convolutional neural network, to predict the function of DNA sequences. This new method allows machine learning researchers to identify some key features that lead to the computer’s decision-making process.

Koo and his colleagues published their findings in *Nature Machine Intelligence* and *PLOS Computational Biology*.

### Shady Communications

Shade avoidance is a vital survival strategy for plants, but it’s a problem for farmers, says CSHL Assistant Professor Ullas Pedmale. When a plant finds itself in the shade, it directs its resources to reach for the light. This shade response limits the density of crop plantings and thus limits yields. Pedmale and his lab discovered a group of proteins called WRKYs that are responsible for stunting root growth in the shade.

Pedmale’s team compared the roots of tomato and *Arabidopsis thaliana* seedlings grown in light to the shorter, less developed roots of plants grown in shade. Postdoc Daniele Rosado and colleagues found hundreds of genes that plants use to respond to stress were switched on in the shade-grown plants—including dozens that encode proteins called WRKYs.

To confirm that WRKYs limit root growth, Pedmale’s team engineered plants in which specific WRKY genes were highly active in light and shade. They found that...
plants with high levels of certain WRKY proteins grew the same stunted roots seen in shade-grown plants, even when provided with plenty of light. The plant’s stems, in contrast, grew at a normal rate.

Pedmale hopes this work will help researchers develop plants that can thrive under more crowded conditions, withstand extreme weather, and pull carbon dioxide out of the air into extensive root systems. The research was published in *Plant Physiology*.

**Corn Evolution**

Doreen Ware, a CSHL adjunct professor and research scientist at the U.S. Department of Agriculture, and her colleagues published the genome sequences of 26 different strains of corn in *Science*. They describe a large portion of the genetic diversity found in modern corn plants and reveal new genetic insights valuable for optimizing the crop for changing climates.

Like a continental landscape, genomic maps have areas that are full of features (like well-mapped cities), whereas others are more like deserts (vast and uncharted). With recent techniques, the team of scientists charted difficult stretches of the genome, even the deserts. These complete genomes allow researchers to locate and study both important crop genes and the nearby regions that regulate their use. Ware notes, “we had little access to the regulatory architecture of corn before.”

The new collection reveals how the corn genome was shuffled over time. Ware says, “Different strains have experienced different environments. For example, some came from tropical environments, others experienced particular diseases, and all those selective pressures leave a footprint of that history.”

Equipped with more detailed maps of the corn genome, scientists have a head start in developing crops for a rapidly changing climate. Ware explains, “The genomes provide broader insights into corn genetics, and this, in turn, can be used to start optimizing corn to grow in future environments.”

**Instant Polymers**

A multi-institutional team of chemists, including CSHL Professor John E. Moses, Nobel laureate K. Barry Sharpless from Scripps Research, and Han Zuilhof of Wageningen University found a way to modify and use a dangerous gas called SOF₄ as building blocks for new products. In a paper in *Nature Chemistry*, they describe a new set of modifiable polymers made from SOF₄.

The team used a type of rapid and reliable chemistry known as click chemistry to “click” molecules together without producing toxic byproducts. The SOF₄ molecule acts as a hub to link together diverse components into a modular family of new—and potentially valuable—drugs and polymers.

The reactions are fast and produce very little waste or dirty byproducts. Moses says, “That’s why click chemistry is great. These polymers could be made in one day. As long as we have the gas, we could do all that chemistry in one day, make a polymer, and post-modify it in one day. That’s incredibly fast.”

This new chemistry will allow scientists to generate a vast new library of polymers, each with its own distinct properties and applications in drug discovery and material science. Moses says, “The opportunity for these polymers, I think, is infinite. There are so many things we can do with it. We’re limited by our imagination.”