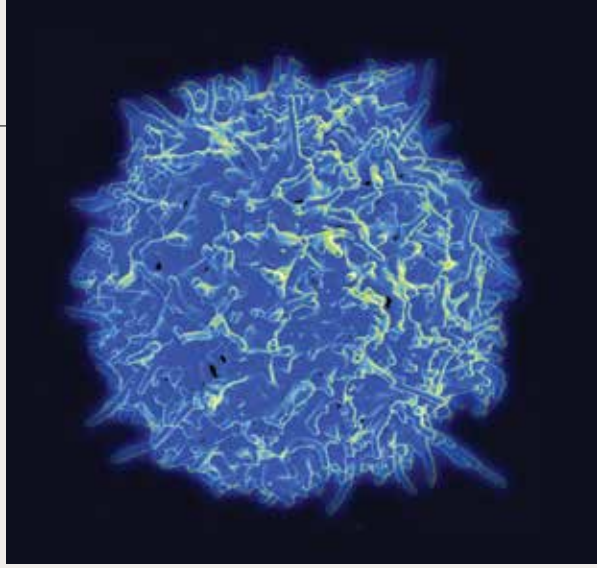


The immune system's T lymphocytes, like this one, need downtime to do their job properly.

T Cell Burnout

Some immune cells function better after taking a break.

EVERY LIVING BEING needs to rest. Even our immune cells enter a dysfunctional state called T cell exhaustion if they're overworked. In fact, this fatigue and the ensuing downtime are important parts of the immune process.

Killer T cells get their name from their function. They target sickened cells involved in chronic infections, killing them before the viruses inside can replicate and spread. But chronic infections can be lengthy, and after a few weeks of sustained action, cellular exhaustion often sets in. The T cells become less effective at slaying their targets and begin producing proteins that prevent them from recognizing infected cells.

One of these proteins is called programmed cell death protein 1, or PD-1. Susan Kaech, an HHMI early career scientist at Yale University, discovered a feedback loop by which PD-1 activation leads to an increase in a protein called FOXO1. FOXO1, in turn, produces factors that promote T cell exhaustion, including more PD-1. Kaech suspected that eliminating FOXO1 might curtail T cells' exhaustion and make them better killers.

What she found was the exact opposite. When her team created mice lacking the *FOXO1* gene, the rodents' T cells did produce less PD-1 compared to animals with the gene. But the cells weren't better at controlling the viral infection. Instead, without the rest afforded them in their exhausted state, the cells died, and viral replication increased.

Kaech concluded that T cells need this respite to function properly. "They have to turn down their response or else they'll get over-activated, and we think that causes the cells to deteriorate and die," she says. "We're starting to appreciate that exhaustion is an important process that is helping to maintain this precious pool of T cells."

The findings, published November 20, 2014, in *Immunity*, could lead to drugs that modulate FOXO1 and help reinvigorate the immune response of patients being treated for chronic viral infections or even cancer. "There may be a point where you can stop the cells from fully entering the exhausted state – where you suppress PD-1, but not so much that the cells die," Kaech says. *–Nicole Kresge*

IN BRIEF

SEEING RED

As a fish swims, nerve cells fire in its brain, sending signals racing along a neural network ending in muscles that make its fins flap and its tail swish. By using a molecule called CaMPARI to permanently mark neurons as they fire, scientists can now watch as signals light up such neural networks in live animals.

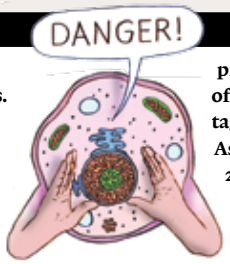
CaMPARI came out of a collaborative project spearheaded by Eric Schreiter, a senior scientist in Group Leader Loren Looger's lab at the Janelia Research Campus. The team started with a protein called Eos, which emits a green glow until it's exposed to violet light. The light changes the molecule's structure, causing it to glow red. By combining Eos with the calcium-sensitive protein calmodulin, the researchers were able to couple Eos's color change to the burst of calcium that accompanies neuronal signaling. The resulting molecule indelibly tags firing neurons with a red glow in the presence of violet light.

"Ideally, we would flip the [violet] light switch on while an animal is doing a behavior that we care about, then flip the switch off as soon as the animal stops the behavior," Schreiter explains. "So we're capturing a snapshot of neural activity that occurs only while the animal is doing that behavior."

The scientists published their results February 13, 2015, in *Science*. Although they are still tinkering with CaMPARI to make it more sensitive and reliable, they've already made it available to scientists on Addgene and the Bloomington Drosophila Stock Center. Janelia Group Leader Misha Ahrens is also distributing CaMPARI-expressing zebrafish.

IMMUNE RALLY CRY

Like a Good Samaritan, a cell that's been attacked by a virus warns neighboring cells to shore up their defenses. These alerts are sent via a family of proteins called interferons, which are produced when surveillance proteins in the infected cell detect a pathogen. Although several different surveillance proteins scout for signs of pathogens, new research shows how the proteins all activate a single molecule called IRF3 to turn on interferon production.



There are three known pathways that trigger type 1 interferon production. In each case, the individual pathway's unique surveillance protein uses its own adaptor protein to relay the message that an invader is present and interferon is needed. Siqi Liu, a graduate student in HHMI Investigator Zhijian "James" Chen's lab at the University of Texas Southwestern Medical Center, noticed that all three adaptor proteins have similar stretches of five amino acids that became tagged with phosphate groups. As the team reported March 13, 2015, in *Science*, the addition of a phosphate molecule to that stretch of amino acids causes the adaptors to activate IRF3.

Now that they know how the interferon pathways converge, Chen and his team are examining them in more detail. Eventually, they hope to develop small molecules that treat immune disorders by interfering with the pathways.

A CAT TALE

A central tenet of biology is that each amino acid in a protein is specified by a three-letter code found in messenger RNA (mRNA). That may be true most of the time, but HHMI Investigator Jonathan Weissman at the University of California, San Francisco (UCSF), along with Onn Brandman at Stanford University and UCSF

Photo: National Institute of Allergy and Infectious Diseases; Illustration: Leif Parsons

Watch a mouse begin to drink thirstily when prompted by a blue light. Go to hhmi.org/bulletin/spring-2015.

Got Thirst? Here's Why

Scientists have pinpointed the neurons in the brain that control thirst.

HUMANS CAN TYPICALLY survive only three to five days without water. But we've all said "I'm dying of thirst" well before we've been waterless that long – albeit with little understanding of the mechanism behind the urge. But now the brain signals that govern thirst are no longer a mystery. A team led by HHMI Investigator Charles Zuker has figured out that the motivation to drink water is controlled by two sets of neurons: one that provokes the stimulus to sip and one that quenches it.

Researchers have long suspected that the signals driving animals to drink originate in the brain's subfornical organ, or SFO. Located outside the blood-brain barrier, where it has the opportunity to directly sense the electrolyte balance in body fluids, the SFO shows increased activity in dehydrated animals. Yuki Oka, a postdoctoral fellow in Zuker's Columbia University lab, took a closer look at the SFO in mice and identified two types of nerve cells: excitatory CAMKII-expressing neurons and inhibitory VGAT-expressing neurons. Using optogenetics, Oka added a light-sensitive protein to the cells in the animals' SFOs, allowing him to selectively activate them with blue light. When he flipped the switch and activated the CAMKII neurons, the rodents drank with gusto.

"You have a water-satiated animal that is happily wandering around, with zero interest in drinking," says Zuker. "Activate this group of SFO neurons, and the mouse just beelines to the water spout. As long as the light is on, that mouse keeps on drinking." Oka showed that the animals became such avid drinkers that they consumed as much as 8 percent of their body weight in water – the equivalent of 1.5 gallons for humans. When Oka used the same technique to stimulate VGAT neurons, thirsty animals immediately stopped drinking and reduced their water intake by about 80 percent. The researchers published their findings online January 26, 2015, in *Nature*.

According to Zuker, the opposing neurons likely work together to ensure animals take in enough water to maintain fluid homeostasis, including blood pressure, electrolyte balance, and cell volume. It remains to be seen whether the same circuit controls thirst in humans; if so, the findings could one day help people with an impaired sense of thirst. *– Nicole Kresge*

The mouse brain contains CAMKII neurons (red) that trigger thirst and VGAT neurons (green) that quench thirst.

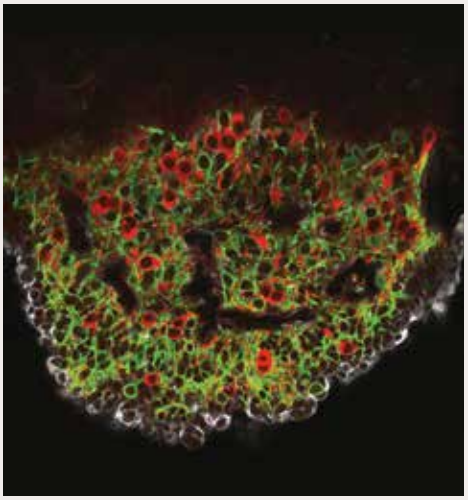


Photo: Zuker lab; Illustration: Leif Parsons

colleague Adam Frost, has discovered an exception to the rule.

Generally, protein synthesis requires three components: an mRNA template, a ribosome to read the template and assemble the protein, and transfer RNA (tRNA) molecules to supply the necessary amino acids. Weissman and his colleagues discovered that sometimes, when protein synthesis stalls, elongation can still continue without the mRNA template. This type of synthesis relies on only two components: a part of the ribosome called the 60s subunit and a protein named Rqc2 that recruits tRNA molecules. With these components, cells will continue to randomly add amino acids to the stalled peptide, with one caveat. Rqc2 recognizes only alanine and threonine (abbreviated "A" and "T"), so it creates what Weissman refers to as "CAT tails."

"This is a mode of protein synthesis that we've missed for 50 years. It's totally unprecedented," says Weissman. He and his colleagues published their findings January 2, 2015, in *Science*. They don't yet know the function of CAT tails, but they have some ideas they're pursuing. For example, the extra amino acids may trigger a stress response in the cell, or they could tag an incomplete peptide for destruction.

HOX PARADOX NO MORE

During development in animals, the Hox gene family directs the formation of segment-specific anatomy along the head-to-tail axis. The genes are responsible for putting the head, thorax, abdomen, and other body parts in the right places. Surprisingly, all Hox proteins bind with high affinity to very similar stretches of DNA, which begs the question: how can Hox proteins find and activate their target genes if all binding sequences look essentially the same? Janelia Group Leader David Stern recently revealed the answer to this long-standing paradox.

Stern's team, in collaboration with Richard Mann's lab at Columbia University Medical Center, figured out that Hox proteins activate genes via weak interactions at previously unrecognized DNA binding sites. The scientists discovered this by showing that a Hox protein called Ubx controls expression of a gene called *shavenbaby* by binding weakly to two enhancer regions on DNA near the gene. These low-affinity binding sites conferred *shavenbaby*'s specificity for Ubx over other Hox proteins.

The findings, published January 15, 2015, in *Cell*, explain why scientists have been unable to predict where Hox proteins bind to DNA simply by looking at its sequence. "They're not regulating genes by binding to the sites that everybody thought they were – they're binding to these sites that don't look like good Hox binding sites," explains Stern.

A TARGET FOR DYSKINESIA

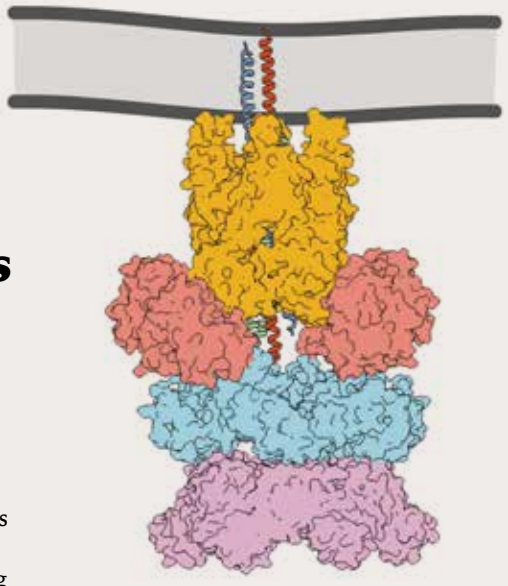
Some human diseases occur episodically in people who appear otherwise healthy. One of these disorders is paroxysmal nonkinesigenic dyskinesia (PNKD). The neurological disease's symptoms – involuntary movements in the limbs, torso, and face – are triggered by coffee, alcohol, and stress. In 2004, HHMI Investigator Louis J. Ptáček at the University of California, San Francisco, discovered the gene that causes PNKD. Now, just over a decade later, he's figured out what the gene does.

To deduce the function of the *PNKD* gene, Ptáček's team raised antibodies against the PNKD protein. This allowed them to isolate PNKD and its binding partners from mouse brain tissue. From these isolates, they discovered that PNKD interacts with two other proteins, called RIM1 and RIM2, that are involved

Chronicle / *Lab Book*

Salvaging SNAREs

Scientists catch a protein-recycling machine in the act.



A cell's NSF complex uses SNAP proteins (orange) to grasp and unwind membrane-embedded SNARE proteins (red and blue helices).

CELLS ARE HUGE proponents of recycling. They reuse everything from tiny electrons to massive molecular complexes. Despite this penchant for salvage, scientists have never been able to see one form of cellular recycling in action – until now. A group led by HHMI Investigator Axel Brunger recently captured a protein in the act of pulling apart a spent cluster of membrane fusion molecules.

"Membrane fusion is a process akin to the merging of soap bubbles into larger ones," says Brunger, a structural biologist at Stanford University. "But that's where the analogy ends, since biological membranes do not merge all that easily." To facilitate the process, cells use membrane-bound SNARE proteins. During fusion, SNAREs located on opposing membranes zip together into a stable complex, linking the membranes. When the two membranes have become one, a protein called NSF recycles the SNARE components.

Brunger's team used a technique called single-particle electron cryomicroscopy to freeze NSF molecules bound to SNARE complexes at several stages and then capture images of them. The resulting snapshots provide near-atomic or better detail of the SNARE disassembly process. Like a series of movie stills, the images show NSF latching onto a SNARE complex, then NSF bound to ATP – the molecule that powers the salvage operation. Yet another image captured NSF after it had finished working, bound to an energy-depleted form of ATP, called ADP.

The SNARE complex resembles a rope with a left-handed twist; the team's images revealed that NSF uses adapter proteins called SNAPs to grasp the "rope" in multiple places. The SNAPs wrap around the SNARE complex with a right-handed twist, suggesting that the disassembly occurs via a simple unwinding motion that frees the zipped SNARE proteins.

The results, published February 5, 2015, in *Nature*, raise other questions the team is eager to pursue. "There is a lot to be done in order to understand the motions and conformational changes needed to disassemble the SNARE complex," says Brunger. "Our electron microscope structures now enable us to design follow-up biophysical experiments to answer these questions at a very deep level." – *Nicole Kresge*

IN BRIEF

in neurotransmitter release. As the scientists reported March 10, 2015, in the *Proceedings of the National Academy of Sciences*, PNKD normally suppresses neurotransmitter release, but the mutant form found in the disease does so less effectively. The result is excessive synaptic transmission, which leads to the involuntary movements experienced by people with the disease.

According to Ptáček, RIM1 and RIM2 may be important targets for developing better medications for PNKD and other dyskinesias, such as those seen in Parkinson's disease.

THE LONG LOST RECEPTOR

In our cells, there is a widely expressed secreted factor called pigment epithelium-derived factor (PEDF), which affects a bewildering array of bodily processes. The cellular receptors that bind PEDF are at the root of many useful activities, such as preventing the formation of new blood vessels, protecting cells in the retina and brain from damage, and stopping cancer cells from growing. Despite considerable effort by scientists in academia and industry, the identity of the receptors had, until recently, remained a mystery. This long-standing puzzle was so intractable that it took HHMI Early Career Scientist Hui Sun and his team at the University of California, Los Angeles, seven years to solve.

"We were initially intrigued by this problem because of PEDF's broad medical value in treating major diseases," says Sun. "But the hunt for the receptor turned out to be an extremely challenging adventure." His team methodically searched for PEDF's binding partner by looking at various native tissues and cells, as well as in databases of molecules with unknown functions. Finally, they found two proteins that fit the bill – PLXDC1 and PLXDC2.

As Sun's team reported December 23, 2014, in *eLife*, these two proteins confer cell-surface binding to PEDF, transduce PEDF signals into distinct types of cells that respond to PEDF, and function in a cell type-specific manner. Because of the critical role of these cell-surface receptors in PEDF signaling, they have high potential as therapeutic targets for cancer and other diseases.

UNIVERSAL PROTEIN SYNTHESIS

The process of gene expression is, at its core, universal. But different organisms use different methods to carry out the "DNA makes RNA and RNA makes protein" recipe. For example, the signals used to recruit ribosomes and start protein synthesis in bacteria are not the same signals used by eukaryotes. Yet much of the structure and function of the ribosome, the molecular machine that translates RNA into proteins, is conserved in both types of organisms. This made HHMI Early Career Scientist Jeffrey Kieft at the University of Colorado Denver wonder if a universal start signal existed that could be recognized by both eukaryotic and prokaryotic ribosomes.

A structured region of an RNA molecule dubbed an internal ribosome entry site (IRES), which is used by some viruses to initiate translation in eukaryotes, fit the bill. Taking a close look at the molecule, Kieft's team discovered that it could also initiate protein synthesis in prokaryotes. This particular IRES, it turns out, binds both bacterial and eukaryotic ribosomes in a similar structure-dependent manner, but the bacterial interaction appears transient and weaker.

The findings, published March 5, 2015, in *Nature*, suggest there might be other naturally occurring structured RNAs that can initiate bacterial protein synthesis. One of Kieft's next steps will be to determine if other such structures do indeed exist, and, if so, what they mean in terms of evolution and gene regulation.