

Synopsis of Research Articles

The Ol' Switcheroo Shows How an RNA Enzyme Splices Itself

DOI: 10.1371/journal.pbio.0030310

Ribonucleic acid (RNA) is a dogma breaker. The “central dogma” of cellular biochemistry mandates that deoxyribonucleic acid (DNA) stores information, and RNA copies this information and uses it to direct the assembly of amino acid building blocks into proteins, such as enzymes. Enzymes catalyze important chemical reactions in the cell, such as the breakdown of glucose or the synthesis of urea.

When biochemists discovered catalytic RNA, they had to ditch the dogma. Because of its structure, it turns out, RNA can act as an enzyme and catalyze reactions. While two strands of DNA tend to zip up into the famous double helix, RNA usually goes solo. The single RNA strand folds back on itself to create myriad tangled arrangements. Some of these arrangements create an active center, the place on the RNA where the enzymatic magic happens. The many RNA enzymes and protein enzymes that use metal atoms to do their job are called metalloenzymes. One example of an important structural motif in RNA metalloenzymes is the group I intron, which can snip itself out of an RNA segment. Understanding exactly how the RNA and the metals interact will help to provide precise answers about how the enzyme really works.

Through X-ray crystallography, researchers have revealed many structural features of group I introns. But X-ray crystallography creates images of the enzyme frozen in time; it does not catch an enzyme in action. In a new study, Joseph Piccirilli, Daniel Herschlag, and colleagues discovered that a particular oxygen atom on a particular nucleotide in a group I RNA must bind to a particular magnesium ion in order for the reaction under study to proceed normally. The oxygen atom is known as the *pro-S_p* phosphoryl oxygen at nucleotide C262 in the intron from the unicellular *Tetrahymena thermophila* protozoan.

Since there's no way to watch the oxygen and metal hook up during the reaction, how do the researchers know they do? The researchers used the powerful techniques of metal ion rescue and atomic mutagenesis. Here's how it worked. They figured out how well the group I intron reaction works with a

normal enzyme. Then, they replaced the oxygen in question with a sulfur atom. The reaction didn't work as well because, by the rules of chemistry, sulfur doesn't like to bind to magnesium. But sulfur does like manganese and cadmium ions. So they replaced the magnesium with one of these other metal ions and measured the reaction. The researchers saw that these other metal ions restored (or “rescued”) enzymatic activity. In short, the enzyme needs a bond where the oxygen and the magnesium are, but the bond doesn't have to be between oxygen and magnesium.

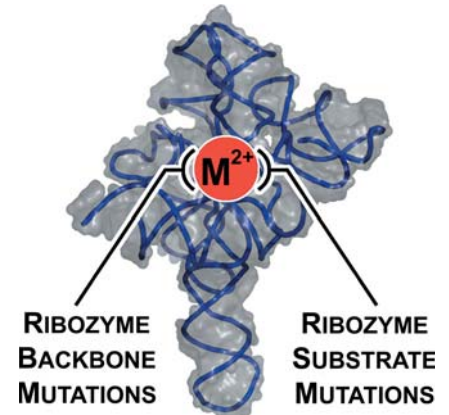
As complicated as that is, plucking out one atom and trading it for another is itself a tricky business. Because most enzymes are made of stubborn amino acids and not nucleotides, atomic mutagenesis can be difficult. And usually when researchers have tried atomic mutagenesis, they've mutated the substrate (the molecule that the reaction acts upon) instead of the enzyme (the molecule that acts). Here, Piccirilli, Herschlag, and colleagues directed the applications of atomic mutagenesis to the molecule that does the work.

To test that a specific oxygen in the intron binds to the magnesium ion, the researchers first had to compile a short list of potential atoms to which the magnesium might bind. By combining literature data from structural models and functional studies with a random sprinkling of sulfur atoms in the intron to find critical oxygen contacts, Piccirilli, Herschlag, and colleagues established a group of specific oxygen atoms to watch.

Genomics Helps Explain Why Some Like It Hot

DOI: 10.1371/journal.pbio.0030317

As warm-blooded creatures, humans and other mammals maintain a relatively stable body temperature that buckles under the stress of intense heat (or cold). When the heat gets too high, we develop fevers and weaken, and our proteins destabilize and degrade—in some cases, such reactions can prove fatal. But some organisms appear to defy nature (as we think of it) by flourishing in extremely high temperatures. The archaeal microbe *Pyrobaculum aerophilum*, for example—originally found in a boiling marine



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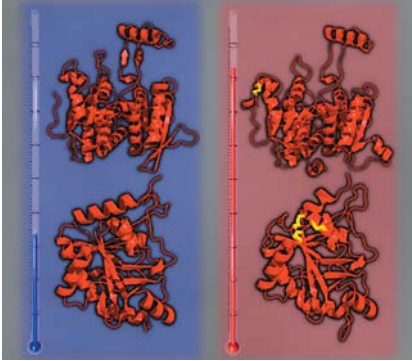
RNA enzymes called ribozymes require metal atoms to function. The site of metal-ribozyme interaction was studied by changing components of both the catalytic center of the ribozyme (the backbone) and its target substrate.

They tried the metal rescue experiment with each of these oxygens, and the only enzyme rescued by the metal switch was the one in which they changed the C262 oxygen to a sulfur. Therefore, they concluded that this specific oxygen atom makes a critical contact with the magnesium ion. The strategy of atomic mutagenesis combined with metal ion rescue can be used to help understand the mechanism of other RNA and protein metalloenzymes.

Houglund JL, Kravchuk AV, Herschlag D, Piccirilli JA (2005) Functional identification of catalytic metal ion binding sites within RNA. DOI: 10.1371/journal.pbio.0030277

water hole in Italy—thrives at ~100 °C (212 °F). Similarly, the bacterium *Thermus thermophilus* grows at temperatures between 48 °C and 85 °C (118–185 °F).

Such organisms are of interest for many reasons—not least of which is to understand the mechanisms that engineer their heat resistance, or thermostability. How do these thermophilic bacteria and archaea manage to maintain active, stable proteins at such high temperatures? In an elegant demonstration of how the



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Some thermophilic bacteria can thrive in extreme heat because their proteins have an abundance of disulfides (yellow, above), covalent bonds between sulfur atoms that improve stability and likely boost heat-tolerance.

ever-growing publicly available genome sequence and protein structure data can be analyzed, Todd Yeates and colleagues identify one answer to this question.

The authors found that proteins from *P. aerophilum* and *T. thermophilus*, along with some other thermophiles, have many disulfides, which are known to improve stability. Disulfides are covalent bonds that form when the sulfhydryl groups (a sulfur and a hydrogen atom) of two spatially proximate cysteines (one

of the 20 amino acid building blocks of proteins) are oxidized. When conditions are right, the two hydrogen atoms are removed by other molecules in the cell dedicated to that purpose, and the remaining sulfur atoms form a bond.

The authors mapped sequences of intracellular genes from 199 prokaryote genomes onto sequence-related proteins with known three-dimensional structures. The resulting structural models reveal when disulfide bonds are likely to form. A pronounced bias was found for disulfides in a set of thermophilic genomes. To prove that these predictions really do form disulfide bonds, the authors solved the structure of one protein from *P. aerophilum*—which was indeed stabilized by three disulfide bonds.

Disulfide bonds form more commonly outside or between cells in multicellular organisms, where the environment is ideal for two cysteines to cozy up and bond in an oxidative extracellular location. The high numbers of bonds observed in these single-cell prokaryotes not only help explain thermostability but also challenge our ideas of how disulfide bonds form. Given the presumed difficulty for disulfides to form in such organisms, the authors set out to look for any proteins that might help explain the mystery. They investigated which proteins are present in the disulfide-

rich organisms as compared with the proteins in other organisms (also known as phylogenetic profiling). The authors discovered that all of the disulfide-rich thermophiles had something else in common: they all encode a protein not seen in other organisms, called protein disulfide oxidoreductase (PDO). As its name suggests, this protein likely plays a key role in the formation of disulfides in these heat-tolerant bugs.

Yeates and colleagues have considerably advanced our understanding of how proteins withstand and remain functional at high temperatures in these thermophilic organisms (via additional stabilizing disulfide bonds). Yet, since this correlation of extra disulfides and the PDO is not common to all thermophiles, it seems likely that this is not the only method employed in heat resistance. Probably a finely tuned concert of different mechanisms works in synchrony to enable thermophiles to flourish in extreme conditions. As the authors show here, it's likely that genome sequence and structure data can help us to uncover these mechanisms.

Beeby M, O'Connor BD, Ryttersgaard C, Boutz DR, Perry J, et al. (2005) The genomics of disulfide bonding and protein stabilization in thermophiles. DOI: 10.1371/journal.pbio.0030309

Islands in the Genome Promote Speciation

DOI: 10.1371/journal.pbio.0030318

Have you ever wondered how the myriad insect forms—beetles, flies, dragonflies, mosquitoes, grasshoppers, ants, wasps, bees, and countless others—evolved? Insects make up 75% of all species known. The large number of insect species is probably a result of a combination of one or more factors: a high rate of formation of new species, or speciation, an ability to adapt to new environments and exploit new ecological niches, and a lower rate of extinction. Speciation, adaptation, and extinction are all controlled by the interplay between genetic and environmental factors. Understanding the genetic changes that lead to the formation of new species is an important area of research in evolutionary biology.

In a new study, Thomas Turner, Matthew Hahn, and Sergey Nuzhdin worked with the malaria mosquito *Anopheles gambiae* to uncover genes that may be driving speciation. *A. gambiae*



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Two forms of the *Anopheles* mosquito, which transmits the malarial parasite, shed light on the genetic changes that prevent related species from producing fertile offspring—a condition of speciation. (CDC)

exists in multiple forms that may be in the early stages of differentiating into separate species; on the other hand, they may be partially differentiated, co-existing races that could give us valuable information on genes responsible for racial differences in mosquitoes. Turner and colleagues focused on two forms, *A. gambiae M* and *A. gambiae S*, that sometimes mate and create hybrid forms in nature. While it's unclear whether the forms can produce fertile hybrid offspring in the wild, the progeny of lab matings appear to have no problems with fertility. This suggests that individuals either naturally prefer to mate with

others of their own form, or that there must be environmental and/or genetic conditions that are not favorable for the survival of hybrid progeny in nature.

To study the genetic underpinnings of speciation, the researchers used DNA microarrays to identify global differences

in the mosquito genomes. Using a combination of gene chips, statistics, and computational biology, Turner and colleagues found that the *M* and *S* genomes differ at just three regions. The researchers suggested that genes present here may be responsible for early speciation. These three “speciation islands” in the genome contain 67 predicted genes. In a preliminary analysis of seven of these genes, Turner and colleagues identified five that are different between the two *Anopheles* forms; these include genes that play a role in a range of cellular processes, including energy metabolism, response to sudden increases in temperature (heat shock), and ion transport across cell membranes. Future work focusing on the 67 genes hypothesized to reside in the divergent regions should yield interesting clues to the identity of genes that drive speciation, and the mechanism by which they do so.

This is a significant finding in the field of speciation research: in terms of methodology, this study shows that DNA

microarrays can be used to identify regions of the genome that are different between two diverging species, allowing researchers to home in on potentially interesting genes. This study also shows that in spite of possible cross-flow of genetic material (natural hybrids between the two forms are found at a low frequency) between two populations, the populations can still be accumulating differences in their genomes—differences that could eventually lead to the formation of new species. Comparing results in *Anopheles* and the well-studied insect model *Drosophila*, in which scientists have also started identifying “speciation genes,” should tell us if similar genes are employed repeatedly in different genera during the formation of new species.

Turner TL, Hahn MW, Nuzhdin SV (2005) Genomic islands of speciation in *Anopheles gambiae*. DOI: 10.1371/journal.pbio.0030285

Speciation Begins, but Doesn't End, with the Twist of a Shell

DOI: 10.1371/journal.pbio.0030330

The coil of a snail shell can be either right-handed (dextral) or left-handed (sinistral), based on whether the shell spirals out clockwise or counterclockwise when viewed from above. Most species are composed entirely of individuals that are one or the other type; in exceptional cases, populations may differ in their handedness, or chirality, but within a single population, all individuals tend to be alike. This makes sense, since the mechanics of reproduction are harder between two individuals of opposite chirality (their genitalia are also reversed), reducing the likelihood that they will successfully mate and produce offspring. Over time, therefore, the rarer type will become rarer and rarer until it goes extinct.

This poses the interesting evolutionary question of how a species of one chirality can give rise to another of opposite chirality. If the rarer types are less likely to reproduce, then how do they ever establish themselves beyond a threshold frequency? If they are able to establish themselves, then is a change in chirality—which is caused by a single gene—enough to isolate them so that they are a new species? A study in this issue by Angus Davison et al. sheds light on the complex interplay of factors that influence evolution in the snail *Euhadra*. Although a single gene does cause a change in chirality, and snails with different chirality are able to mate only with great difficulty, there is nevertheless almost free gene flow between them. Other factors must ultimately become involved to cause speciation.

The 22 species of *Euhadra* are land-dwelling natives of Japan, and include five sinistral and 17 dextral species. Using mitochondrial DNA analysis to construct a family tree, the authors showed that



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Even though snails with reversed shell spirals have trouble mating (their genitalia are also reversed), gene exchange occurs freely between two forms in a population, suggesting that speciation requires other factors.

the sinistral species compose a distinct branch, indicating that this feature arose only once in the history of the genus. How did the first sinistral shell types arise, and why didn't they gradually evaporate from the gene pool? One possibility is “reproductive character displacement,” in which a new feature that directly affects mating, such as sinistral shell chirality, decreases the likelihood that its owner will mate with snails of other, closely related, species that live nearby. While their dextral brothers or sisters waste valuable resources in such unsuccessful interspecific pairings, the few sinistral individuals engage in fewer, but more productive, matings exclusively with their own kind, thus increasing their numbers despite the odds stacked against them.

To test this hypothesis, Davison et al. constructed a model that took into account a variety of factors, including

population density, the proximity of other species, and the maternal inheritance pattern of shell chirality (the direction of a snail's shell is determined so early in development that it is governed not by its own genes, but by its mother's). The surprising conclusion is that the last factor, the unusual mode of inheritance, allows for near free gene flow between the two forms within a population, even if the two forms are themselves almost unable to mate. The reason is that the offspring of a sinistral mother could itself be sinistral, even if it contains entirely dextral genes. Its offspring, though, might include dextral snails, because its own dextral genes determined their shell chirality.

Their model indicated that new chiral types are able to arise, in spite of there being fewer suitable mates, if there is reproductive character displacement. They cannot be considered new species, however, because of the gene flow between them. Reproductive character displacement can account for the speciation of sinistral *Euhadra* only under a complex set of conditions. Interspecific mating would need to be common among the dextral snails. High population density helps, since it allows those with the rare new form to find each other more easily. But gene flow between left and right forms would preserve the population as a single species, unless other factors, such as difference in habitat use or geographic separation, increased the isolation of the two forms. This argues against so-called “single-gene speciation,” and shows that the creation of a new species requires more than a simple twist of fate.

Davison A, Chiba S, Barton NH, Clarke B (2005) Speciation and gene flow between snails of opposite chirality. DOI: 10.1371/journal.pbio.0030282

A Recipe for Self-Renewing Brain

DOI: 10.1371/journal.pbio.0030307

In all the hullabaloo about stem cells, nobody has noted their uncanny similarity to pizza dough. You can divide either into two or four or eight identical pieces, but that doesn't determine what kind of cell or pizza you're going to make. But once you let a cell grow hundreds of nuclei, or you pile on the pepperoni, you're on your way to making a skeletal muscle fiber or a pepperoni pizza. If you want a white blood cell or an all-veggie pie, you're out of luck. The commitment to becoming a certain cell type is called differentiation.

Stem cells in living organisms can multiply without differentiating, preserved by molecular signals in special niche environments; without these signals in the petri dish, they differentiate. Pluripotent mouse embryonic stem (ES) cells, a special type of stem cell with the potential to develop into many different cell types, are an exception. Because they divide symmetrically, the scads of artificially grown ES cells are all the same. This leads researchers to wonder: what conditions in the body keep stem cells from differentiating, why are ES cells the only kinds that don't differentiate in the petri dish, and how can scientists create undifferentiated tissue-specific stem cells in the lab?

In a new paper, Austin Smith and colleagues developed a method to produce symmetrical divisions of mouse brain stem cells derived from ES cells. Their novel method creates an on/off switch for differentiation of tissue-specific stem cells: they can multiply without differentiation, and they can also become normal brain cells. The authors also managed to cultivate the brain stem cells without re-creating the rarefied neurosphere, the highly specialized environment or microenvironment in which the body grows its own brain stem cells.

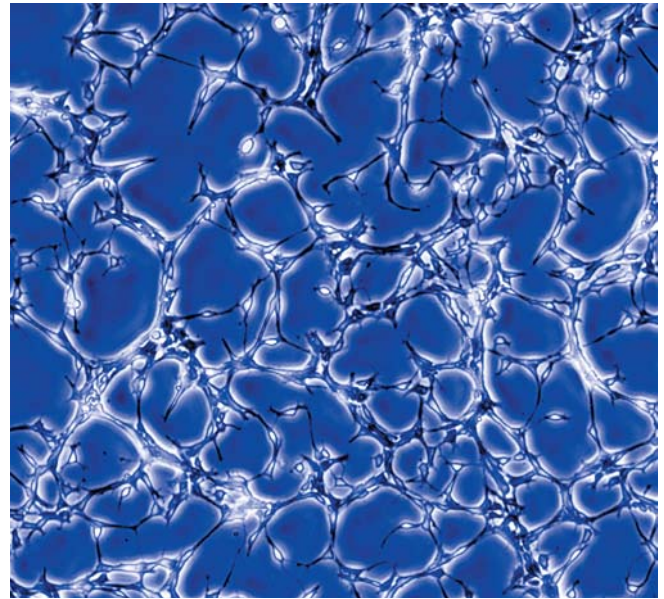
Many scientists believe that in the body, these microenvironments prevent stem cells from differentiating. Neurospheres, for example, contain some undifferentiated brain stem cells floating in a broth of differentiating cells. One feature of the neurosphere is that a very low percent of cells are brain stem cells. In fact, neurospheres have so few of these cells that scientists have a hard time even observing them. But by cultivating brain stem cells outside the neurosphere, the scientists showed that a complex microenvironment may not be necessary. To grow their stem cells, Smith et al. combined epidermal growth factor (EGF) and fibroblast growth factor (FGF), two small proteins that bind to stem cells and promote growth.

Enlisting Genomics to Understand Flu Evolution

DOI: 10.1371/journal.pbio.0030302

Last October, as Americans started lining up for flu shots, news broke that 48 million vaccine doses had been contaminated. With 100 million people considered at high risk and fears of a potentially deadly avian flu epidemic on the horizon, the shortage caused long lines, allegations of price gouging, and a new bill to bolster the nation's anemic vaccine manufacturing base.

Influenza A viruses are RNA viruses that infect humans, pigs, horses, and birds, both wild and domestic. Flu infection relies on a viral glycoprotein, hemagglutinin (HA), that binds to receptors on a host cell and allows the virus to be internalized. If antibodies produced by host immunity recognize viral antigens (on the surface of the HA protein), HA binding is inhibited and infection prevented. A virus's best chance of gaining the upper



DOI: 10.1371/journal.pbio.0030307.g001

A new technique allows researchers to culture brain stem cells in unlimited numbers and then induce differentiation into mature neurons or glia by changing growth factors.

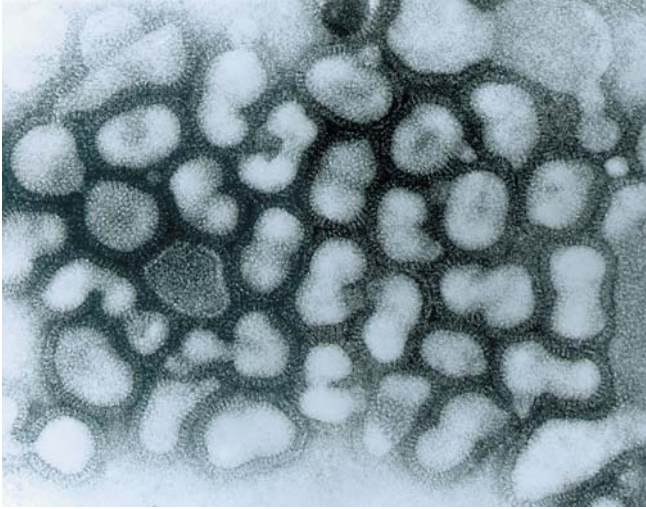
Previously, scientists had grown brain stem cells with FGF. Upon removing FGF, the cells failed to differentiate and become mature. The cells that Smith et al. grew, in contrast, became mature cells upon removal of the growth factor cocktail. They observed both neurons and astrocytes, the two types of cells into which the brain stem cells mature.

In the future, scientists may use this new technique to produce large quantities of the cells to study their basic properties and also to explore their value for modeling neurodegenerative afflictions, including Huntington disease, Parkinson disease, and Alzheimer disease. Additionally, these cells may clinch the debate of whether doctors will be able to use stem cells directly to repair brain damage.

Conti L, Pollard SM, Gorba T, Reitano E, Toselli M, et al. (2005) Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. DOI: 10.1371/journal.pbio.0030283

hand in this evolutionary game of cat and mouse is to change its HA in a way that eludes antibody recognition. Typically the mutations are minor and the virus's antigens conserved enough for the host body's immune system to recognize them. On occasion, influenza can acquire an antigenically novel HA subtype, becoming a virulent pandemic strain that completely escapes immune surveillance and kills millions. Minimizing the effect of yearly influenza outbreaks—by developing effective matched vaccines—depends on predicting which flu strains are likely to evolve.

Toward this end, Eddie Holmes and colleagues took the global approach afforded by genomics to explore the forces underlying viral adaptations. They found multiple flu strains



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A transmission electron micrograph of the influenza A virus. New evidence suggests that flu viruses can rapidly reshuffle genetic material and mutate into new strains capable of widespread infection. (CDC/Dr. Erskine Palmer)

circulating in the population at the same time, and a more complex evolutionary pattern than previously thought. They also showed that co-circulating viruses can exchange genes in a way that creates antigenically novel, epidemiologically significant strains—a process that humans may facilitate by simultaneously hosting more than one strain.

They analyzed the genomes of 156 influenza A viruses (serotype H3N2) collected by New York State public health officials between 1999 and 2004 in search of global patterns of viral evolution. Using the flu virus genome sequences produced

at the Institute for Genomic Research (TIGR), funded by a National Institute for Allergy and Infectious Diseases (NIAID) initiative, the authors grouped the viral sequences according to sequence similarity. They also included partial flu sequences obtained from other studies in their analysis. These data are the initial output of the first large-scale effort to completely sequence influenza genomes. While most of the virus genomes sampled after 2002 fell into one group—which the authors called clade A—there were also other clades circulating at different times (called clades B and C).

Gene trees, or phylogenies, constructed for each of the virus's eight genes all diverged according to their respective clades, except one—the HA gene. The HA gene cluster grouped all the clade A viruses that emerged after 2002 as well as both the clade B and C viruses from the same time period and viruses from multiple locations (in Asia, Australia, Europe, and North America).

Altogether, these results indicate that different viral strains had circulated in the same populations until 2002 and then the clade A and C viruses acquired a common HA gene from clade B through reassortment. While reassortment between co-circulating human influenza strains has been previously described, this study is the first to examine in detail a reassortment event leading to an epidemiologically significant outcome, the emergence of the “Fujian” strain in the 2003–2004 season. Though it's not yet clear how variant clades manage to persist alongside dominant strains, the fact that they do suggests the influenza virus has multiple adaptive tools at its disposal. Luckily, the tools of genomics should help predict what evolutionary paths the virus might take and help in the process of selecting the most promising vaccines to contain it.

Holmes EC, Ghedin E, Miller N, Taylor J, Bao Y, et al. (2005) Whole-genome analysis of human influenza A virus reveals multiple persistent lineages and reassortment among recent H3N2 viruses. DOI: 10.1371/journal.pbio.0030300

For Insulin Signaling Pathways in Flies, Size Matters

DOI: 10.1371/journal.pbio.0030320

Insulin or insulin-like proteins signal developing animals to grow. After a meal, the body creates insulin, allowing an organism to grow and compete with other organisms for available food. When food is scarce, insulin levels remain low. Only small organisms with low metabolic needs will survive the potential famine. Scientists can study how genes involved in insulin signaling affect development by mutating a gene and seeing what happens to the adult. This useful method, called gene knockout, provides insight into the specific relationship between a gene and its physical manifestation, or phenotype. By using the knockout method, scientists can observe how the growth of an organism responds to fluctuations in insulin signaling levels.

In a new study, Alexander Shingleton and colleagues used a temperature-sensitive mutation in an insulin-receptor gene to discover how alterations of insulin signaling in the fruitfly *Drosophila*

affect different stages of fly development. At one stage, the researchers discovered, insulin signaling influences total development time, at another it influences body size, and at a third stage, it influences only organ size.

So when do developing flies need insulin? The researchers found that low insulin signaling during very early development extends total development time. Then the larvae reach their critical size, the watershed moment in insect development when larvae commit to becoming pupae. After critical size, reduced insulin signaling no longer delays development but instead leads to petite flies with petite organs. When the larvae become pupae, however, reduced insulin signaling simply creates smaller organs. Because developmental time, body size, and organ size each display different responses to reduced insulin signaling activity, these features may evolve independently, the authors reasoned.



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Experiments in the fruitfly show that insulin signaling controls body size, as well as organ size and total development time, by affecting cell growth and proliferation.

Shingleton and colleagues used flies with mutant insulin-receptor genes whose protein products are partially inactivated at high temperatures. During different stages of the flies' development, the scientists cranked

up the heat (from 17 °C to 24 °C) and watched what happened to their bugs. Using this temperature-sensitive insulin-receptor gene, the researchers found that, besides affecting development time, insulin signaling also plays a role in the differential growth rates of different organs. By tracking three organs on male flies, Shingleton and colleagues discovered that the genitals are less sensitive to reduced insulin signaling than either the wings or the maxillary palps, olfactory components of the mouth. The authors also found that

insulin signaling affects cell size and cell number differently. While slightly reduced insulin signaling shrinks cell size, highly reduced insulin signaling lowers cell number without affecting cell size. By incorporating the effects of reduced insulin signaling into the *Drosophila* development process, the authors constructed a model of *Drosophila* development that explains the various roles played by the insulin-signaling pathway during development.

Because the new study alters genes during development, it provides the

details of when and how a developing animal requires insulin. Future fly studies may reveal why organs have individual responses to insulin signals, what other signaling pathways play a role in development, and how insulin came to influence so many different features of the developing fly at different times.

Shingleton AW, Das J, Vinicius L, Stern DL (2005) The temporal requirements for insulin signaling during development in *Drosophila*. DOI: 10.1371/journal.pbio.0030289

To Mosquitoes, People with Malaria Smell Like Dinner

DOI: 10.1371/journal.pbio.0030306

Malaria is a misnomer. People used to believe that poisoned or “bad air,” the translation of the Italian phrase “mal aria,” caused disease. In the 19th century, when parasitologists figured out that single-celled parasites cause malaria, they didn’t bother to change the disease’s name. Experimenters proved that these parasites need a host organism to survive—so they can’t be transmitted through air—and that the hosts, mosquitoes, carry the parasite to humans. Researchers were optimistic that if they could find a disease’s cause, they could also find the cure. Kill the mosquitoes and eradicate malaria. And with the advent of DDT and less environmentally harmful insecticides, potent anti-malarial drugs, and international funding in the late 20th century, eradication of malaria seemed imminent.

But that expectation underestimated the flexibility of living creatures. Mosquitoes acquired resistance to insecticides while the parasites acquired resistance to anti-malarial drugs. Worse, the aggressive eradication campaign skipped over vast regions of the globe, especially sub-Saharan Africa.

Malaria remains a devastating problem in Africa for several reasons. Environmental conditions provide an amenable atmosphere for both *Plasmodium falciparum*, the most dangerous form of the parasite, and the *Anopheles gambiae* mosquito, the most effective vector. Also, many countries in sub-Saharan Africa lack the infrastructure to protect their citizens from malaria. Given the overwhelming scope of malarial infection in Africa, new understanding of the disease will help epidemiologists devise targeted anti-malarial strategies.



DOI: 10.1371/journal.pbio.0030306.g001

Mosquitoes are most attracted to children infected with malarial parasites in the gametocyte stage (pictured above). The *Anopheles* mosquito ingests gametocytes during its blood meal. (CDC/ Dr. Mae Melvin)

A new study conducted in Western Kenya by Jacob Koella and colleagues analyzed mosquito behavior to discover how it facilitates the transmission of malaria. The research determined that mosquitoes are more attracted to people infected with transmissible malaria than to either people infected with non-transmissible forms of the disease or uninfected people. To measure the attraction of the mosquitoes, the researchers set up a chamber of infected mosquitoes surrounded by tents containing the study participants. A device called an olfactometer wafted the odors of each participant toward the mosquitoes. Researchers measured which smell most attracted the hungry bugs.

This question had long stalled scientists because of contradictory and indirect evidence. Sweat, breath odor, and high body temperature all increase mosquitoes’ blood lust, and no previous study had isolated the variable of malarial infection.

To control for the natural variation in how attractive mosquitoes found each participant, Koella et al. compared the number of mosquitoes that were attracted to infected people to the number of mosquitoes that were attracted to those same people after they were no longer infected. The researchers found that in general, an individual attracted more mosquitoes when infected with transmissible malaria. This demonstrates that malaria, in addition to causing fever, vomiting, headache, and sometimes death, causes more mosquito bites. The biting mosquitoes will then pick up the parasite and spread it to other people.

As another control, the researchers compared infection with a non-transmissible form of the parasite to infection with the transmissible form and to no infection. A mosquito can pick up the malaria parasite only when in its sexually reproductive stage. The transmissible parasite, known as a gametocyte, multiplies in the mosquito’s belly before traveling to the mosquito’s salivary glands and, eventually, to the blood of the next human victim. But the malaria parasite has a complicated life cycle that also includes non-transmissible asexual stages. Koella and colleagues found that these parasitic forms, unlike the sexually reproductive form, did not make humans more attractive to mosquitoes.

Previous to the recent study, malaria researchers had proved that mosquito biting rates greatly influence the spread of malaria. Koella and colleagues showed that the parasite itself increases these biting rates when it is ready for a new host.

Lacroix R, Mukabana WR, Gouagna LC, Koella JC (2005) Malaria infection increases attractiveness of humans to mosquitoes. DOI: 10.1371/journal.pbio.0030298

How Fruitflies Know It's Time for Lunch

DOI: 10.1371/journal.pbio.0030332

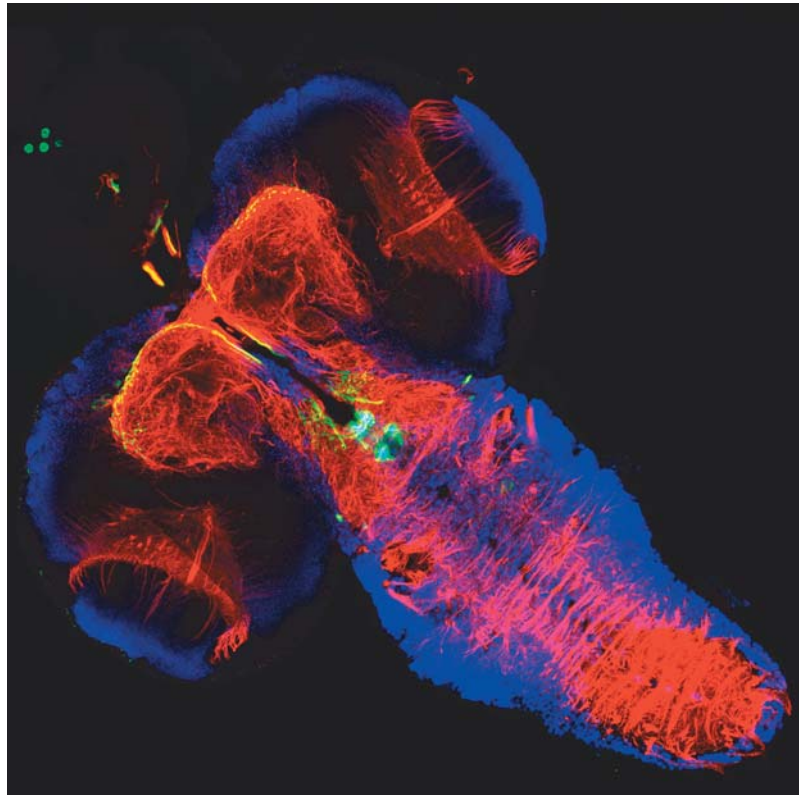
To control what you eat and when, your nervous system must coordinate a laundry list of signals: internal signals contain information about energy level, food preferences, and metabolic need, while external signals relay information about the quality of available food, determined by its smell and taste. Scientists studying the fruitfly *Drosophila* have traced the path of olfactory signals beginning with chemical receptors in the mouth, which set off neurons that signal the antennal lobe of the central nervous system. From here, the electrical stimulation zooms toward the so-called mushroom body, a mushroom-shaped cluster of neurons involved in olfactory processing. Less is known about the gustatory signals, which begin both in the mouth and in the pharynx and aim toward the subesophageal ganglion region of the fly's brain. How olfactory and gustatory signals influence feeding patterns remains murky.

In a new study, Michael Pankratz and Christoph Melcher used genetic analysis to gain insight into the adult and larval neural networks that use taste information to regulate eating. Specifically, they found that several types of neurons responsible for coordinating taste signals express the gene *hugin* (*hug*), a gene linked to abnormal eating activity and expressed in only the subesophageal ganglion. By altering *hug* expression, the researchers uncovered the gene's behavioral influence: *hug*-expressing neurons influence a fly's decision to sample new food sources. The researchers also proposed that *hug* proteins play a role in hormone-triggered growth, an important consequence of adequate feeding.

To begin their investigation, Melcher and Pankratz analyzed the DNA from flies with abnormal eating behavior. One group of these flies shared a mutant *klumpfuss* (*klu*) gene, normally responsible for encoding a protein transcription factor. Because neural transcription factors control production levels of other neural proteins, the researchers used DNA microarrays to compare gene expression in normal flies to that in *klu* mutants. Any *klu*-controlled genes expressed at different levels in *klu* mutants might contain clues about the neural circuitry modulating feeding behavior.

Using microarrays, Melcher and Pankratz discovered that mutant fly larvae overexpress the *hug* gene, which is known to encode at least two neural proteins related to growth signaling. The researchers then investigated which signals influence *hug* expression by exposing larvae to either high or low food levels. Because both starved and sugar-fed flies express little *hug*, the researchers inferred that *hug* levels do not solely signal internal energy requirements but respond to internal and external signals carrying information about the quality of food. The researchers also noted that the finicky *pumpless* (*ppl*) mutants, which have a feeding defect similar to *klu*, overexpress *hug*.

Behavioral studies confirmed that too much *hug* reduces food intake and leads to stunted growth, while too little stimulates eating. Melcher and Pankratz selected a group of flies and blocked the synapses of their *hug* neurons to inhibit the neurons'



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Microarray, neuroanatomical, and biochemical analyses identified taste-sensitive neurons that help regulate feeding behavior in fruitfly larvae.

activity. In contrast to control flies, which start feeding on a novel food source only after an evaluation phase (they wait a while before initiating feeding), the experimental flies started eating new food right away. These *hug* neurons may help flies decide whether or not to eat a new food source.

Larvae express *hug* in only about 20 neurons, all located in the subesophageal ganglion. The axons of some of these *hug* neurons extend into the ring gland, a crucial metabolism and growth organ in flies. Other axons contact the protocerebrum, a structure close to brain centers for learning and remembering odors. A third set of these axons extend to throat muscles—which is surprising because most subesophageal ganglion neurons have no connection to motor function. All together, these few *hug* neurons can signal structures controlling growth, feeding, and learning and memory.

Besides linking *hug* neurons to brain centers that regulate taste-related feeding behavior, the study also raises questions about how the nervous system prioritizes internal and external signals. How hungry must flies be to overcome taste aversion? How do the competing neural networks of taste and hunger signals decide whether the fly will eat? Future studies pairing behavioral and genetic analysis may begin to reveal answers to these open questions.

Melcher C, Pankratz MJ (2005) Candidate gustatory interneurons modulating feeding behavior in the *Drosophila* brain. DOI: 10.1371/journal.pbio.0030305

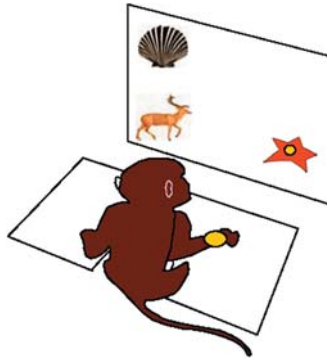
Clocking in Pillow Time without the Pillow

DOI: 10.1371/journal.pbio.0030308

If you snooze, you lose those uncomely grayish-brown crescents below your eyes. If you don't snooze, you lose a lot more. The body can't fight off infection, the muscles can't regenerate as quickly, the mind can't learn new words, and the eyes can't focus on the road. You also gain things: a bad mood and increased risk for diabetes, high blood pressure, and heart problems. Indeed, the effects of sleep deprivation can be so serious that some sleep scientists liken lifetime sleep debt to a heavy backpack: every sleep hour missed adds an extra pound to your pack until it weighs you down.

For people without time for a daily eight hours in the sack, drugs that counteract the effects of sleep deprivation could serve as substitutes. In a new study, Sam Deadwyler and colleagues have explored this possibility by giving dog-tired rhesus monkeys a drug shown to improve the functioning of alert brains. They found that sleepy monkeys taking the drug performed tasks better and had increased metabolic activity in several regions of their brains. This suggests that the cognitive effects of sleep deprivation can be reduced chemically.

The researchers kept the monkeys awake for 30 to 36 hours by playing music and videos, keeping the lights on, and interacting with them: all the annoyances that can also keep humans from sleeping. To determine the drug's effect on drowsy monkeys, Deadwyler and colleagues used a behavioral test called Match-To-Sample, which measured both accuracy of memory and speed of recall. In the behavioral test, the monkeys saw a simple image flash on a screen. For a variable amount of time, the monkeys had to remember the image. Then, they had to select the correct image from a group of others shown on the monitor simultaneously. When monkeys correctly selected the original image with a cursor, they got a squirt of juice in their mouth as reward. The researchers measured how long they could keep the screen blank between the first and second images without affecting the monkeys' performance. They found that if the monkeys were tired, they couldn't remember the first image for long as they could when they were alert. But with the drug, the sleep-deprived monkeys did at least as well as alert monkeys.



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By training monkeys on a classic "match to sample" task, researchers show that ampakine drugs can alleviate the cognitive defects associated with sleep deprivation.

The drug, labeled CX717 (Cortex Pharmaceuticals), acts on AMPA receptors, protein structures on the surface of neurons. When these receptors bind to the neurotransmitter glutamate, they transduce excitatory signals by opening an ion channel. Ampakines including CX717 make the activated channel stay open longer when glutamate binds. More ions pass through the channel, creating a stronger signal when nerve cells are activated. The ubiquity of these receptors makes them good targets for drugs that increase general cognitive functioning.

The researchers used a technique called positron emission tomography, or PET, to gain insight into CX717's neurobiological role. The PET signal reflected the distribution and rate of metabolism of ingested radioactively labeled glucose in the monkeys' brain cells. By measuring regional brain glucose metabolism, the researchers determined that for sleep-deprived monkeys, glucose metabolism drops off in brain areas previously associated with memory tasks—namely, the prefrontal cortex, the dorsal striatum, and the medial temporal lobe. However, when sleep-deprived monkeys took the drug, they showed heightened glucose metabolism in these same brain regions. The researchers compared these results to suggest a biological basis for the drug's effects.

Previous studies have shown that caffeine and amphetamine can reduce the deleterious cognitive effects of sleep deprivation. But as anybody who has indulged one latte too many knows, caffeine and other powerful stimulants have limited usefulness. These potentially addictive chemicals can distort thinking just as they can enhance it. Because CX717 has a different biochemical action, it may be more beneficial than stimulants for counteracting the cognitive effects of sleep deprivation. But that doesn't mean we should throw away our pillows and blankets just yet: sleep deprivation affects both body and mind.

Porrino LJ, Daunais JB, Rogers GA, Hampson RE, Deadwyler SA (2005) Facilitation of task performance and removal of the effects of sleep deprivation by an ampakine (CX717) in nonhuman primates. DOI: 10.1371/journal.pbio.0030299

A Genetic Link to Obesity: The Numbers Don't Add Up for *GAD2*

DOI: 10.1371/journal.pbio.0030321

Obesity is a leading cause of preventable death and is often linked to type II diabetes and heart disease. Being a complex trait, obesity is likely caused by the interplay of multiple environmental factors and many genes. Common genetic differences between individuals within a region of Chromosome 10 have previously been associated with obesity. This region contains several genes with the potential to be directly involved in the

disease. One of these genes, *GAD2*, has been the subject of many studies. A new study by Michael Swarbrick, Björn Waldenmaier, Christian Vaisse, and their colleagues takes a new look at *GAD2* and provides strong evidence that the gene might not be as relevant to obesity as previously thought.

GAD2 encodes a protein (called GAD-65) involved in the production of GABA, a neurotransmitter involved in a variety of brain functions, including appetite

stimulation and energy consumption. Studies in mice have shown that increased levels of GABA result in hunger and overeating. In healthy mice, the levels of *GAD2*, and hence, GABA, are controlled, making sure that the balance between weight gain and loss is maintained. A 2003 study of a French population found that three genetic mutations in and around the *GAD2* gene occurred at a high level in individuals with obesity. The 2003 study, conducted by different researchers,



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Scientists believe that genetic mutations in a specific region in Chromosome 10 play a role in obesity and have studied one gene, *GAD2*, intensively. But a new study finds no evidence linking *GAD2* mutations with obesity.

was also published in *PLoS Biology*. When Swarbrick et al. surveyed German, Caucasian-American, and Canadian populations for this genetic correlation,

however, they found no statistically significant link between obesity and any of the mutations.

There are many possible reasons why different studies may show different results: ethnic differences between populations, as well as behavioral and dietary differences, could account for varying results when it comes to studying a trait as complex as obesity. Also, studies that seek to show an association between genetic differences and complex diseases rely heavily on the statistical power of their tests, which depends on the number of subjects involved. Swarbrick et al. have not only studied 2,359 German, 729 US, and 1,137 Canadian subjects, but also conducted a “meta-analysis”—a statistical analysis of a collection of individual studies—of their data and the previously published data from 1,221 French subjects. Meta-analyses help identify patterns from multiple individual studies that may not

be visible in any one study alone, and also help rule out chance differences that may be apparent in one single study. In this case, the meta-analysis showed that when the results from French subjects are put together with the results from other ethnic populations, there is no evidence for a link between changes in *GAD2* and obesity.

Although *GAD2*'s role in controlling appetite made it an exciting candidate for a link to obesity-related conditions, Swarbrick et al. show that the numbers simply don't add up. The search for serious obesity gene contenders in this region of Chromosome 10 is all set to continue—and attention can now turn to several other potential gene candidates located nearby.

Swarbrick MM, Waldenmaier B, Pennacchio LA, Lind DL, Cavazos MM, et al. (2005) Lack of support for the association between *GAD2* polymorphisms and severe human obesity. DOI: 10.1371/journal.pbio.0030315

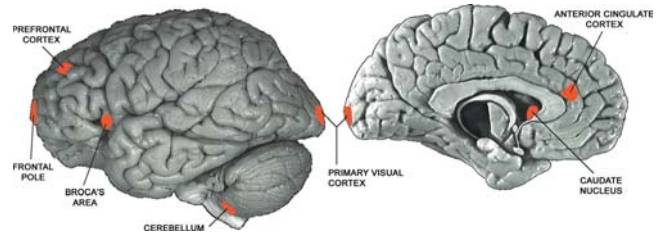
Gene Expression in the Aging Brain

DOI: 10.1371/journal.pbio.0030313

No matter how healthy a life one leads, no person has managed to live much longer than a century. Even though the advances of the modern age may have extended the average human life span, it is clear there are genetic limits to longevity. One prominent theory of aging lays the blame on the accumulation of damage done to DNA and proteins by “free radicals,” highly reactive molecules produced by the metabolic activity of mitochondria. This damage is expected to reduce gene expression by damaging the DNA in which genes are encoded, and so the theory predicts that the most metabolically active tissues should show the greatest age-related reduction in gene expression. In this issue, Michael Eisen and colleagues show that the human brain follows this pattern. A similar pattern—which, surprisingly, involves different genes—is found in the brain of the aging chimpanzee.

The authors compared results from three separate studies of age-related gene expression, each done on the same type of DNA microarray and each comparing brain regions in young versus old adult humans. In four different regions of the cortex (the brain region responsible for higher functions such as thinking), they found a similar pattern of age-related change, characterized by changes in expression of hundreds of genes. In contrast, expression in one non-cortical region, the cerebellum (whose principal functions include movement), was largely unchanged with age. In addition to confirming a prediction of the free-radical theory of aging (namely, that the more metabolically active cortex should have a greater reduction in gene activity), this is the first demonstration that age-related gene expression patterns can differ in different cells of a single organism.

The authors found a similar difference in age-related patterns in the brain of the chimpanzee, with many genes down-regulated in the cortex that remained unchanged in the cerebellum. However, the set of affected cortical genes



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Gene expression data from three microarray studies of primate brains were used to identify genetic changes associated with aging in humans and chimps. Different brain regions in both primates undergo distinct age-related changes in gene expression.

was entirely different between humans and chimps, whose lineages diverged about 5 million years ago. The explanation for this difference is unknown, but the finding highlights the fact that significant changes in gene expression patterns, and thus changes in many effects of the aging process, can accumulate over relatively short stretches of evolutionary time.

These results raise a number of questions about age-related gene expression changes, including whether metabolically active non-brain tissues display similar patterns of changes, and whether the divergence between human and chimp patterns was the direct result of selection, or was an inevitable consequence of some other difference in brain evolution. The patterns seen in this study also provide a starting point for understanding the network of genetic changes in aging, and may even reveal targets for treatment of neurodegenerative diseases.

Fraser HB, Khaitovich P, Plotkin JB, Pääbo S, Eisen MB (2005) Aging and gene expression in the primate brain. DOI: 10.1371/journal.pbio.0030274

Misty Watercolor Memories, Biochemically Speaking

DOI: 10.1371/journal.pbio.0030304

Eyewitness testimony has a unique ability to convince juries. The attorney asks the witness to identify the guilty party. The witness points to the defendant, the crowd gasps, and the judge pounds her gavel, demanding order in the court. The jurors casually scribble something in their notes, and everybody knows that the fate of the accused has been sealed. But how reliable is a witness's memory, especially after rehearsing the testimony ad nauseam with a team of lawyers? When a witness presents testimony, is she really remembering the event, or is she remembering something she remembered? Does the initial memory remain intact, or does it degrade like a copy of a copy?

The status of witness testimony in court is just one reason neuroscientists want to understand the biochemical underpinnings of memory formation. Consolidation, the process of new memory formation that takes place in the hippocampus, requires certain proteins. Reconsolidation, the reactivation of these memories in the amygdala, requires a different set of proteins. In the past, neuroscientists hypothesized that reconsolidation might allow old and new memories to link up. A new study by Cristina Alberini and colleagues provides evidence that when rats link new memories to old, the molecular basis of this process actually resembles consolidation.

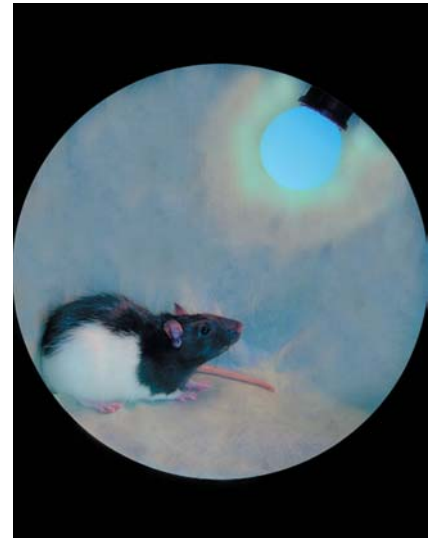
To manipulate lab rat memories, the researchers used constructions called inhibitory avoidance apparatuses. The first apparatus had two tiny rooms: a well-lit safe room and a pitch-black electric-shock room. Rats spent ten seconds in the first room, the researchers flipped on a light, and the rats entered the shock chamber.

Alberini and colleagues knew that the rats had formed a new memory when they hesitated to enter the dark room.

Rats then entered a second apparatus decorated differently from the first apparatus. The safe room smelled of perfume, the walls displayed striped wallpaper, and the floor was made from smooth plastic. For rats in the second apparatus, the researchers flipped on a light but did not let the rats pass into the shock room. Alberini and colleagues deduced that the rats had compiled their memories of both the first and second apparatuses when they hesitated to enter the second dark room during a final test.

The researchers found that rats injected with anisomycin, a drug that inhibits protein synthesis, could not form a new memory of the second apparatus and sometimes forgot the first. This showed that, as predicted, both the formation of new memories and the reconsolidation of old memories require protein synthesis. The researchers demonstrated the distinction between the processes of consolidation and reconsolidation by showing that rats require a certain protein in the hippocampus only for memory consolidation and the same protein in the amygdala only for reconsolidation.

Using a combination of proteins that took advantage of the differences between consolidation and reconsolidation, the researchers inhibited either the rats' consolidation mechanism or the reconsolidation mechanism. Then, Alberini and colleagues tested the rats' ability to link their memory of the first apparatus to their exposure to the second. Upon repeated trials, the rats with blocked reconsolidation pathways successfully linked memories



DOI: 10.1371/journal.pbio.0030304.g001

Training rats to associate light with a traumatic experience helped researchers identify the mechanisms that allow the brain to link new memories with recollections. (Sophie Tronel and Ryan Corces-Zimmerman)

of both apparatuses, while the rats with blocked consolidation pathways did not. Therefore, the consolidation pathway, and not the reconsolidation pathway, plays a role in memory linkage.

As a cautionary word, the researchers emphasized that their results applied to the fear-based memories created by the electric shock. Future studies may reveal if other types of memory yield the same results.

Tronel S, Milekic MH, Alberini CM (2005) Linking new information to a reactivated memory requires consolidation and not reconsolidation mechanisms. DOI: 10.1371/journal.pbio.0030293