Resolving the Family Tree of Placental Mammals

Liza Gross | DOI: 10.1371/journal.pbio.0040111

Natural selection was particularly inventive in the appendage and accessory department during the evolution of placentals—an expansive category of mammals that bear live, fully developed offspring. A placental might sport webbed wings, a prehensile tail, flippers, fangs, tusks, cloven hooves, paws, claws, floppy ears, horns, or any number of other specialized structures. While such morphological characteristics shed light on evolutionary relationships, they can also confound classifications because animals might independently acquire the same traits without sharing a common ancestor.

With an ever-growing repository of genome sequence data, scientists have increasingly turned to molecular techniques to help resolve evolutionary relationships. Several recent molecular analyses offer support for placing recent placentals into four major groups: Afrotheria (mostly African species, including elephants and aardvarks), Xenarthra (New World species such as armadillos and sloths), Laurasiatheria (includes carnivores, whales, and horses), and the newly reclustered Supraprimates (includes rodents and primates). Supraprimates and Laurasiatheria are further grouped together as sister taxa in a larger group called Boreotheria. Molecular approaches have also tried to resolve the hotly debated issue of where to draw the base of the placental tree, though no consensus has emerged. These studies arrived at these conclusions by analyzing different datasets of nuclear and mitochondrial genes under different models of DNA sequence evolution.

But such molecular approaches have their own limitations, as genomes can also contain confounding features (called homoplasies), similar elements that look alike but do not represent common ancestry. In a new study, Jan Ole Kriegs, Jürgen Schmitz, and their colleagues used a different molecular strategy to infer the evolutionary history of placentals, relying on retroposons to signal kinship. Unlike mitochondrial or nuclear genes, retroposons are virtually free from homoplasies. They are reliable markers for inferring evolutionary history, the researchers explain, because their integration into the genome is random—making it highly unlikely for the same element to integrate independently into a conserved region of the genome (called an orthologous position) in two different species. In addition to finding "significant support" for the previously identified divisions, they offer strong support for placing Xenarthra—armadillos and their kin—at the base of the placental tree.

Using specialized computer software, Kriegs et al. searched the mouse, dog, and human genome databases for the presence (or absence) of retroposons. From the 237 candidates identified in the scan, they designed PCR primers (a technique to identify and generate sufficient amounts of specific sequence for analysis) to amplify the equivalent sequences from organisms representing each placental superorder. When size differences between amplified and original sequences (indicating the presence or absence of a retroposed element) occurred within orthologous genome sites, the researchers repeated the analysis with loci from different taxa. (For example, an element might be present in all boreotherian species, but absent in afrotherians and xenarthrans, which diverged before the insertion occurred.) Twenty-eight loci showing size shifts within orthologous sequences were identified for further sequence analysis.

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In this painting of the evolution of placental mammals, the blue time line connects two globes of different earth ages symbolizing the time scale of evolution. Major divergences of mammalian lineages are symbolized by members of crown groups. (Painting: Tomas Schmitz)

Kriegs et al. next studied the presence/absence patterns of these loci to determine how the various placental representatives were related. This analysis yielded markers that provided solid evidence for the divergence of several superordinal groups, as well as the base branch on the placental tree. Four markers occupied the same orthologous location in every species sampled except for the opossum, demonstrating the power of retroposons to reveal evolutionary splits, even as long as 100 million years ago. Eleven markers were present in all sampled supraprimates and laurasiastherians but not in Afrotheria or Xenarthra, supporting the supergroup Boreotheria. The separate laurasiatherian and supraprimate classifications were also reinforced by the identification of markers found exclusively within both groups.

Evidence that Xenarthra represents the first split in the placental tree comes from the finding that two markers are present in both Boreotheria and Afrotheria but not in Xenarthra. This suggests that Xenarthra represents a sister group to all the other placental mammals (collectively referred to as Epitheria)—a hypothesis proposed by classical morphological taxonomists.

Interestingly, other molecular techniques have come to different conclusions, with a 2001 molecular study of nuclear and mitochondrial DNA reporting that Afrotheria was likely the earliest diverging group. But Kriegs et al. make a strong case that retroposons provide a reliable metric for identifying the likely inhabitants of the basal branch of the placental tree—Xenarthra. With this technique added to their genomics toolbox, scientists can continue to investigate this and other questions concerning placental evolution as more xenarthran and afrotherian sequence data become available. By combining high-throughput bioinformatics with high-throughput diagnostic lab techniques, this study provides a valuable framework for homing in on the true genetic footprints of evolutionary history.

Kriegs JO, Churakov G, Kiefmann M, Jordan U, Brosius J, et al. (2006) Retroposed elements as archives for the evolutionary history of placental mammals. DOI: 10.1371/journal.pbio.0040091

Note Added in Proof

The version of this synopsis that was first published online on March 14, 2006, has been replaced by this, the definitive version, which contains an updated figure caption and figure credit.

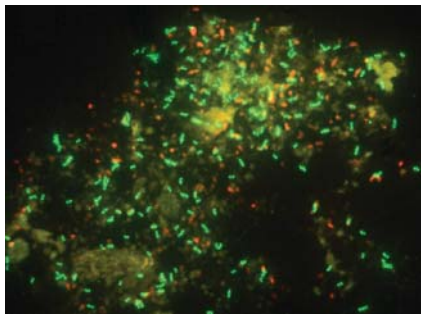
Genomics Sheds Light on Metabolism of Cryptic Marine Microbes

Liza Gross | DOI: 10.1371/journal.pbio.0040123

In 1977 Carl Woese and George Fox expanded our appreciation of microbial diversity by analyzing the genetic sequence of a molecule (ribosomal RNA) found in all cells. They discovered that species previously classified as bacteria, called methanogenic bacteria, possessed unique enzymes and an unusual metabolism based on reducing carbon dioxide to methane. These traits were foreign to both "uber" domains of life, Eurkaryota and Bacteria, prompting Woese to create a new category, which he called Archaebacteria (*archae* means ancient in Greek), acknowledging a metabolism that would have suited the putative conditions on earth over 3 billion years ago.

Archaeal groups have been found in a wide array of habitats—from boiling sulfur pits, salt marshes, and hydrothermal vents to frosty Antarctic surface waters, mud flats, and freshwater habitats—yet less than 0.1% of expected species have been characterized.

In a new study, genetic analysis offers clues to the fundamentals of archaeal life and some insight into how these organisms can exist in such diverse environments. Steven Hallam, Edward DeLong, and their colleagues enlist genomics techniques to identify the pathways used by the marine sponge symbiont *Cenarchaeum symbiosum* to accomplish life's most essential processes: energy metabolism and carbon assimilation. And by comparing the *C. symbiosum* genome sequence with sequences extracted from environmental samples collected from diverse ocean habitats, they show that



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The bright green cells represent the archaeal sponge symbiont, *C. symbiosum*, stained with a fluorescently labeled *C. symbiosum*-specific DNA probe. The red cells represent surrounding bacteria also found in the sponge host tissue.

planktonic Crenarchaeota share many of the same genetic components.

Many archaeal species can use inorganic compounds (rather than sunlight, like plants) as an energy source for carbon synthesis, earning them the unwieldy name of chemolithoautotroph. Several lines of evidence suggest that planktonic Crenarchaeota, significant components of the marine ecosystem, assimilate carbon in this way and that they might use ammonia (NH_3) as an energy source, since they inhabit ammonia-rich Antarctic waters and are associated with high nitrite concentrations. (Nitrite is a by-product of ammonia oxidation.)

To search for genetic clues to carbon and energy metabolism in Crenarchaeota, the researchers extracted *C. symbiosum* DNA from its host sponge and constructed a DNA library for sequencing the symbiont's genome. Hallam et al. then searched for representative genes linked to pathways associated with autotrophic carbon assimilation. They found many components of two pathways: the 3-hydroxypropionate cycle and the reductive tricarboxylic acid (citric acid) pathway (TCA). Both cycles involve a multistep series of chemical reactions that convert inorganic compounds—in this case, carbon dioxide—into organic carbon molecules. Though some components of the 3-hydroxypropionate cycle were missing in *C. symbiosum*, enough elements (including core proteins) were found to support a modified version of this pathway for carbon assimilation, using carbon dioxide.

In eukaryotes, the TCA cycle links the oxidative breakdown of carbon compounds with biosynthesis and energy metabolism. In prokaryotes, the process is reversed, with the oxidation of inorganic compounds (such as carbon dioxide) providing the means for carbon assimilation. Again, though some TCA components were missing, Hallam et al. found evidence suggesting that *C. symbiosum* could use partial TCA reactions to produce biosynthetic precursors. It's possible that other genes take the place of the missing components or that the TCA and 3-hydroxypropionate pathways overlap.

The researchers next searched for genes that might play a role in generating energy from ammonia oxidation (also called nitrification because ammonia is converted to nitrite). The *C. symbiosum* genome contains many genes associated with

nitrification in bacteria, including genes that encode the subunits of ammonia monooxygenase, which catalyzes the first step in converting ammonia to nitrite. The researchers could not find evidence for several proteins that function downstream in this pathway, however, suggesting that the symbiont uses alternative mechanisms to effect nitrification. Supporting this possibility, the researchers found candidate genes that might take the place of some of these missing elements, as well as others that could protect the cell from the toxic nitrites generated by ammonia oxidation.

How did the genes identified here compare with planktonic Crenarchaeota gene sequences? To find out, Hallam et al. searched GenBank (the National Institutes of Health genetic sequence database) and an environmental database containing gene sequences collected from the Sargasso Sea and other ocean waters for similar sequences. Many components of both the 3-hydroxypropionate and the TCA cycle were found in the environmental database. And each of the *C. symbiosum* genes studied here were most closely related to sequences from planktonic Crenarchaeota—suggesting that even though these archaeal lineages evolved under different selective pressures, they rely on similar metabolic strategies.

Overall, Hallam et al. argue that, though the forms show significant divergence at the nucleotide level, *C. symbiosum* and planktonic Crenarchaeota share "striking" similarities in the identity and organization of their genes. And with gene sequences linked to fundamental processes like carbon assimilation and energy metabolism in *C. symbiosum*, researchers can probe parallel processes in marine Crenarchaeota—an endeavor that will likely reveal the vital role these once-enigmatic organisms play in the carbon and nitrogen cycles of marine ecosystems.

Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, et al. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. DOI: 10.1371/journal.pbio.0040095

The Neural Persistence of Memory: Retention Begins While You're Still Awake

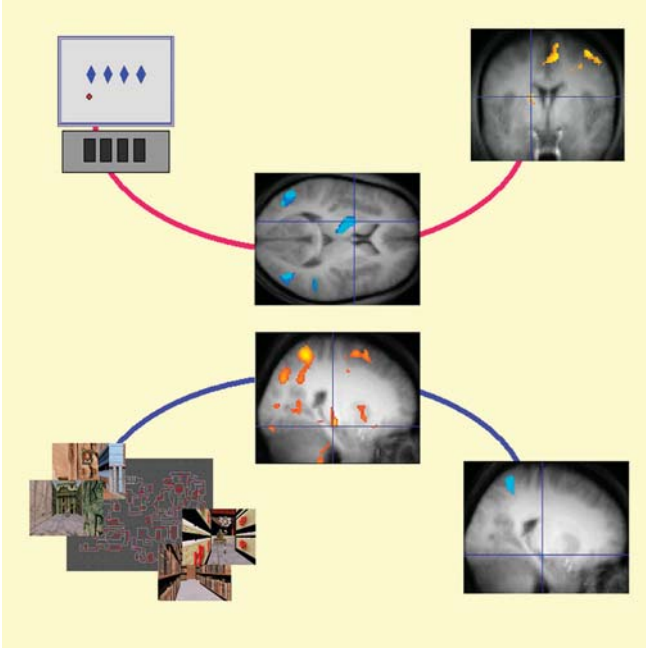
Liza Gross | DOI: 10.1371/journal.pbio.0040116

There's some unwritten law of stadium parking that says after any event some fraction of hapless souls must perform an embarrassing reenactment of *Dude, Where's My Car?* It might seem like a simple thing to remember until you consider that the brain must often process and retain new memories while simultaneously tending to several unrelated cognitive tasks. Though it's not exactly clear how the brain processes a recent memory, evidence suggests that a good nap during an event might prevent parking mishaps. Many studies have shown that brain regions activated while learning a task are reactivated during sleep, suggesting that this "offline" processing facilitates memory retention. But when does the memory consolidation process begin?

Studies in rodents and monkeys have shown that the same neuron ensembles activated during the practice phase of a task continue to be activated for several minutes immediately after exposure to a new task. This suggests that delayed activation in the brain represents a step in the memory storage process.

In a new study, Phillippe Peigneux, Pierre Orban, Pierre Maquet, and their colleagues used functional magnetic resonance imaging to test this possibility and probe the fate of recent memories in the human brain. The researchers asked individuals to perform two separate tasks associated with different memory systems, and found that regional brain activity associated with learning a task persisted and evolved while participants completed an unrelated task. The learning-dependent changes in regional brain activity they observed while individuals were awake echoed those seen during sleep. This offline activity may act as a placeholder, maintaining newly acquired information until it gets transferred to long-term storage during the memory consolidation process.

The researchers chose spatial and procedural tasks that are known to induce post-training brain activity in learning-related sectors during sleep. Each task engages a different brain sector—the spatial task depends on the hippocampus while the procedural task relies on cortical and subcortical regions—allowing the researchers to distinguish each task's post-training brain activity from activity associated with practicing a different task.



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After participants learned to perform either a procedural or a spatial task, learning-dependent, regional brain activity persisted and evolved with time, suggesting the neural integration of recent memories.

For the spatial task, participants navigated a path through virtual space; for the procedural task, they indicated under which of four position markers a dot appeared by rapidly pressing a keystroke. For the unrelated "oddball task," participants lay in the scanner and mentally counted the deviant sounds embedded in a monotonous soundtrack. These oddball sessions occurred immediately before a

task—providing baseline brain activity—immediately after a 30-minute training session, and again after a 30-minute rest period. A short behavioral test followed the last oddball session, then participants were scanned a fourth time while performing their task to identify brain regions associated with each task. Two weeks later, individuals were tested on the alternate task, so the researchers could compare post-training modulated brain activity associated with each task.

Brain responses to the oddball task were significantly higher immediately after training on the spatial task than they were in the pre-training session. Delayed post-training activation (after the break) also remained significantly higher in the hippocampus and other brain regions associated with spatial navigation. The pattern for the procedural task was similar but followed a different time course. Brain activity in cortical and subcortical regions associated with task performance decreased immediately after training but then showed a delayed increase, above pre-training levels, in learning-related brain sectors.

For both tasks, modulated offline activity showed a tighter coupling with other brain regions associated with learning each task following the training period; this coupling occurred immediately after training for the spatial task and after a 45-minute delay for the procedural task. The researchers went on to relate these post-training, task-dependent, regionally specific changes to post-training performance.

The relationship between behavioral performance and functionally significant brain activity changes suggests that this offline activity plays a role in maintaining and processing newly acquired memories. Moreover, the researchers argue, these neural correlates of memory maintenance—persistent and reorganized neural activity that occurs while you're alert and tending to other matters—operate in different brain regions at different times to process distinct types of memories. It remains to be seen whether persistent neural traces continue after memories are consolidated. So you may not need that nap to remember where you parked your car after all—but it wouldn't hurt to jot down the location, just in case.

Peigneux P, Orban P, Balteau E, Degueldre C, Luxen A, et al. (2006) Offline persistence of memory-related cerebral activity during active wakefulness. DOI: 10.1371/journal.pbio.0040100

The Key to Longevity? Having Long-Lived Parents Is a Good Start

Liza Gross | DOI: 10.1371/journal.pbio.0040119

Many studies show that tweaking a single gene can extend life span in the worm and other model organisms. That's nice for them, you may say, but what about humans? It stands to reason that if manipulating a key gene can increase longevity in these animals, humans may well harbor genetic variants, or alleles, that confer some protective advantage to the same end.

In a new study, Gil Atzmon, Marielisa Rincon, Nir Barzilai, and their colleagues followed this logic to look for genetic clues to longevity in a group of 214 Ashkenazi Jews who have passed or nearly reached the century mark. Since centenarians are not prone to cardiovascular disease, diabetes, and other age-related disorders, the researchers reasoned, it's likely that they possess protective genotypes that increase the likelihood of reaching a ripe old age. And if this is the case, these genotypes should occur with higher frequency in centenarians than they do in the rest of us. And, indeed, the researchers found a specific genetic profile, or genotype, that was associated with cardiovascular health, lower incidence of hypertension, greater insulin sensitivity, and longevity.

Ashkenazi Jews were recruited for the study because genetic and historical evidence suggest that the population descended from a founder group of just 30,000 or so people 500 years ago. Populations derived from a very narrow founder group tend to be more genetically homogenous than other populations, simplifying the challenge of linking a genotype to its physical manifestation (phenotype). Since longevity runs in families, the researchers could circumvent the obvious problem with finding a control group age-matched to the centenarians by recruiting children of the centenarians and then finding other Ashkenazi Jews the same age to serve as the controls.

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Exceptionally long-lived individuals share a common genetic feature: they posses a SNP in the promoter of a gene involved in lipid metabolism.

Each participant received a physical examination and had blood drawn for genotyping and measuring levels of cardiovascular disease markers, including insulin, cholesterol, triglycerides, high-density lipoproteins (HDL, the "good" cholesterol), low-density lipoproteins (LDL, the "bad" cholesterol), and concentrations of two lipoprotein components, called apolipoproteins (APO). In a previous study, the researchers had found that

centenarians' lipoproteins were larger than normal, so they also measured LDL and HDL particle size, too.

To identify genotypes that might be associated with a longevity-conducive genotype, they focused on single nucleotide polymorphisms (SNPs) in 36 genes involved in lipoprotein metabolism and other pathways linked to cardiovascular disease. This analysis revealed a polymorphism in a gene with a clear pattern of age-dependent frequency: *apolipoprotein C3 (APOC3)*. The polymorphism replaces an A (adenine) nucleotide with a C (cytosine) in the gene's promoter region, where transcription is initiated. The frequency of the *APOC3* polymorphism (CC) occurring in both copies of the gene was 25% among centenarians, 20% in their offspring, and 10% in controls.

APOC3 proteins are a major component of very low density lipoproteins (VLDL, another type of bad cholesterol) and also occur in HDL. Recent reports have linked elevated APOC3 protein levels (linked to an insulin-resistant form of the gene) to increased risk of cardiovascular disease, along with various *APOC3* polymorphisms, which did not change APOC3 levels. Given the pattern observed here and both genes' role in lipoprotein metabolism, the researchers expected that this genotype would have a protective effect and that carriers would have a favorable lipoprotein profile. And, indeed, all participants carrying the *APOC3* CC polymorphism had better triglyceride and cholesterol levels, as well as the beneficial particle size. This favorable profile corresponded to about 30% lower APOC3 serum levels.

And unlike the recently reported insulin-resistant *APOC3* genotype, this genotype corresponds to greater insulin sensitivity. Since insulin inhibits *APOC3* transcription, this may explain why APOC3 serum levels were lower in individuals carrying two copies of the allele. These individuals also had a significantly reduced prevalence of hypertension.

Altogether, the statistical associations between *APOC3* and longevity and the significant links between favorable lipoprotein-related traits and longevity strongly suggest the genotype's multifaceted contribution to cardiovascular health and longevity. Functional studies can now address whether the *APOC3* polymorphism directly influences APOC3 levels and the observed benefits or flags a nearby SNP that causes these effects. The genetic pathways driving longevity are unknown, but it seems clear that lipoprotein metabolism plays an important role—the favorable lipoprotein profiles reported here fall in line with studies of Japanese and Italian centenarians as well. By combining genotype studies of "exceptionally aged" individuals with functional studies of the identified genes, researchers can continue to tease apart the molecular agents of aging—and begin to develop strategies to ease the inevitable slide into our twilight years.

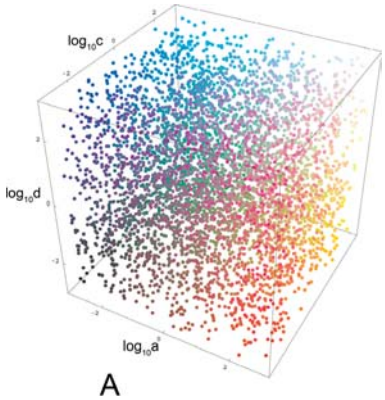
Atzmon G, Rincon M, Schechter CB, Shuldiner AR, Lipton RB, et al. (2006) Lipoprotein genotype and conserved pathway for exceptional longevity in humans. DOI: 10.1371/journal.pbio.0040113

Mutations Change the Boolean Logic of Gene Regulation

Richard Robinson | DOI: 10.1371/journal.pbio.0040064

It is easy to think of a gene acting like a light bulb, switching either on or off, remaining silent, or being transcribed by the RNA-making machinery. The region of DNA that controls the gene's output is called its regulatory region, and in this simple (and too simplistic) scenario, that region would act like a simple on–off switch.

But the regulatory regions of real genes are more complex, and act more like molecular computers, combining the effects of multiple inputs and calibrating the gene's output accordingly. The inputs are the various molecules that affect gene activity by binding to sites in the regulatory region. These molecules combine their effects in complex ways. Sometimes the gene remains silent unless both are present. Sometimes they are additive, such that the output when two factors are present is twice the output when only one is present. Sometimes they cancel each other out—in the presence of either, the gene is transcribed, but in the presence of both, it is not.



DOI: 10.1371/journal.pbio.0040064.g001

Each point on this graph represents three parameters that describe the binding affinities of RNAP, LacI, and CRP to the *lac* operon.

Thus, the regulatory region acts as a Boolean logic function, whose simple ANDs, ORs, and NOTs combine to determine the output of the gene. In a new study, Avi Mayo, Uri Alon, and colleagues show that mutations in the regulatory region affect this logic function in a simple and well-studied genetic system, the *lac* operon in *Escherichia coli* bacteria, whose suite of genes regulate metabolism of lactose.

The authors began by creating multiple strains of bacteria with mutations in the binding sites for the two regulators of the gene, called CRP and LacI, that respond to cyclic AMP and IPTG, an analog of lactose. They analyzed the effect of these mutations on the rate of gene transcription in the presence of varying concentrations of the two inducers. Previously, the authors showed that the function of the unmutated regulatory region was intermediate between a pure "AND gate" (in Boolean parlance) and a pure "OR gate": that is, at certain concentrations the first regulator AND the second were needed, but at others, one OR the other sufficed. In the mutated strains, they found that some mutations replicated this behavior, while others switched the regulatory region to a more purely AND or purely OR gate, independent of concentration. Some mutations left the regulator almost like a simple light

switch, whose on-or-off state depended almost entirely on one, but not the other, regulator.

Next, they developed a mathematical model that links the binding strengths of the regulators for each mutation (the "inputs" of the "regulatory function") to the gene output. Based on this model, they propose that point mutations in this system cannot create all of the 16 possible two-input gates described by Boolean logic. For instance, since both regulators stimulate gene activity, no simple mutation is likely to switch the system to an "AND NOT" gate, in which one input can stimulate only when the other is not present.

The authors suggest that applying this kind of logic analysis to genetic "circuits" may aid in the design of artificial genetic systems, and in understanding more complex gene regulatory regions. With only 30,000 genes, it is clear that humans and other complex creatures must depend on exquisitely regulated gene expression to develop and adapt to environmental changes. The findings in this study support the growing appreciation that, from bacterium to baleen whale, complexity is highly dependent on fine-tuning gene regulation.

Mayo AE, Setty Y, Shavit S, Zaslaver A, Alon U (2006) Plasticity of the *cis*-regulatory input function of a gene. DOI: 10.1371/journal.pbio.0040045

The Sound of Dinner

Françoise Chanut | DOI: 10.1371/journal.pbio.0040107

Bats are not blind, but many rely primarily on sounds to navigate and forage. For the big brown bat, a common denizen of treetops and old house attics, dinner starts at dusk and consists of flying insects—mostly beetles—caught on the wing. As they soar from their caches, bats probe the night with piercing ultrasonic cries, listening for guidance cues in the echoes returned by reflective surfaces. In a wide-open space, the cries dissipate before they bump into the surroundings, and the only perceptible echoes come from objects—potentially winged insects—that pass within a few yards of the bat's large ears. But in a more complex environment, such as a wooded field, a bat's challenge is to hear the faint echo of a tasty morsel over the noise bouncing off trunks, leaves, and every blade of grass.

In a recent study, Cynthia Moss and her colleagues detected rhythm changes in the cries of bats hunting in cluttered versus open environments. The researchers' observations show that bats have an uncanny ability to adjust their sensory apparatus—in this case, their vocal performance—to their perception of the environment, a phenomenon known as active listening.

Big brown bats can emit cries of variable pitch, length, and repetition rate, which generate a wealth of details for their finely tuned ears to ponder

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Echolocating bats adjust their vocalizations to catch insects against a changing environmental background.

in the returning sounds: an echo with a sliding pitch suggests a moving object, while intensity flickers signal a fluttering wing. Subtle discrepancies in pitch spectrum reveal an object's size and texture, while time delays between cry and echo translate into distance. Having thus identified an appetizing prey, the bat zeroes in on it with cries of increasing frequency and decreasing duration. Its final pounce is guided by rapid spitfire known as the final buzz.

Moss and her colleagues focused on the last few seconds before the final buzz, a time when bats emit clusters of two or more short pulses separated by silent intervals ten to 25 milliseconds long. The researchers hypothesized that these quick, repetitive pulses, known as "sonar strobe groups," might be particularly well suited to resolving a small object from a complex background. They filmed and recorded individual big brown bats launching on a tethered mealworm inside a dark, bedroom-sized chamber. A synthetic plant hanging at various distances from the worm created clutter. Simultaneous sound and high-speed video recordings of the attack allowed the researchers to match cries and echoes precisely with the stages of the hunt and the bats' wing-beat pattern.

In an empty chamber, bats attempted capture and succeeded every time, after a mere two-second hunt. But clutter muddied their hearing. When the worm hung closest to the plant (ten centimeters), the bats made for the prey only half of the time, and overwhelmingly failed, presumably because they couldn't distinguish worm from foliage. As the distance between prey and clutter increased to 40 centimeters, attempts and success rates increased while hunting times decreased from one minute back to a few seconds.

Clutter also altered the cries' sequence, most dramatically within the last second of the hunt. Bats curtailed their final buzz 3-fold and spent more time strobing in the presence of clutter, an indication that strobing is indeed used to increase resolution power. In addition, they did not quicken the pace of their strobe groups as fast: the silent intervals between pulses progressively shortened in both situations, but slowly and only to a third of their original length in clutter, whereas they quickly dropped by half in an open-room hunt. Interestingly, bats emitted strobe groups at any stage of flight, contrary to former theories that linked sound production to wing beat.

The slower rhythm change of the sonar strobe groups in clutter is a further hint that the bats use these sound clusters to improve their perception of complex echoes. Still, the fact that there is useful information in a signal does not mean that the information is used. Ultimately, experiments that disrupt the strobe groups will be needed to prove their utility.

Moss CF, Bohn K, Gilkenson H, Surlykke A (2006) Active listening for spatial orientation in a complex auditory scene. DOI: 10.1371/journal.pbio.0040079

One Signal, Multiple Pathways: Diversity Comes from the Receptor

Liza Gross | DOI: 10.1371/journal.pbio.0040131

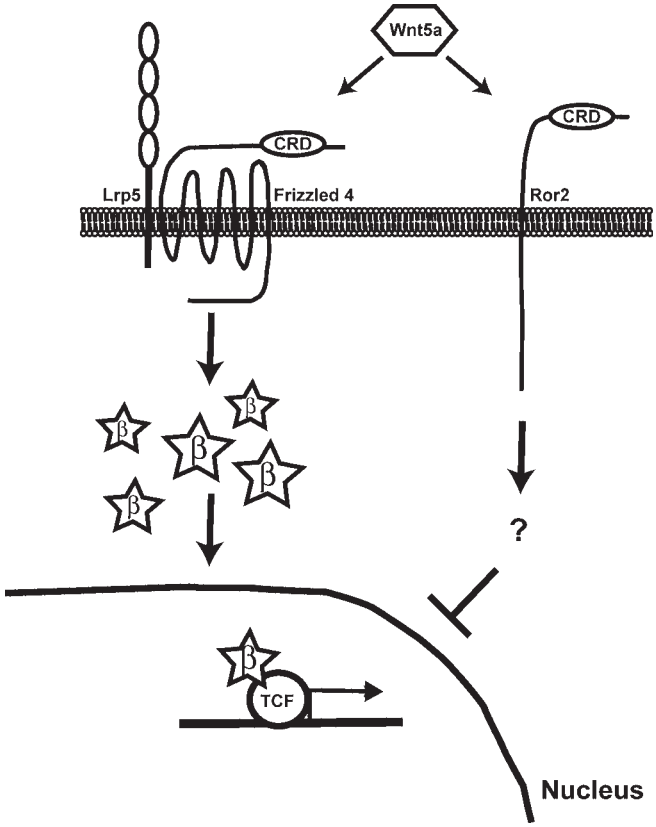
Type "Wnt" into Google Scholar, and you'll get nearly 72,000 hits, revealing the pivotal role this widely conserved family of signaling proteins plays in development and disease. Wnt proteins trigger complex signaling cascades that regulate cell growth, migration, differentiation, and many other aspects of development with the help of numerous interacting components. In the best-understood, "canonical" pathway, Wnt signaling molecules (called ligands) bind simultaneously to two coreceptors on the cell surface (Frizzled and LRP), allowing β-catenin proteins to stabilize (avoid destruction), enter the nucleus, associate with the transcription factor complex TCF/LEF, and activate genes involved in cell survival, proliferation, or differentiation. Inappropriate activation of β-catenin has been linked to several types of cancer.

Wnt ligands have also been implicated in several alternative, "noncanonical" pathways, challenging researchers to figure out how proteins that appear so similar at the sequence level can produce such different results. Studies in frogs and zebrafish embryos suggest this diversity derives from engaging multiple pathways, with Wnt5a, for example, triggering an intracellular calcium release that activates calcium-dependent signaling molecules. It's also possible that Wnt5a signals through other receptors (besides the canonical Frizzled receptor) with a Wnt-binding domain, such as the receptor tyrosine kinase-like orphan receptor 2 (Ror2). But because isolating Wnt ligands in a soluble form has proven difficult, scientists have been forced to resort to indirect methods of studying the mechanisms of Wnt studies, which often provided varying and conflicting results.

In a new study, Amanda Mikels and Roel Nusse have developed a technique to purify the Wnt5a protein and directly investigate its contribution to different pathways. They show that soluble Wnt5a proteins can both inhibit and activate the canonical pathway, depending on which combination of receptors is expressed on the cell surface. When Wnt5a interacts with Ror2, the canonical Wnt/β-catenin pathway is inhibited; when it engages Frizzled and LRP, the β-catenin pathway is activated.

The researchers modified a Wnt purification technique previously established in their lab to harvest Wnt5a proteins from cells engineered to overexpress the mouse *Wnt5a* gene, and confirmed the identity of the protein by examining a key part of its amino acid sequence. Having confirmed the identity of the protein, they compared Wnt5a's capacity to mediate signaling in cells expressing different combinations of the Ror2, Frizzled, and LRP surface receptors. They also examined Wnt5a's capacity to modulate signaling by Wnt3a, which is known to activate the canonical pathway.

First, the researchers tested the possibility that Wnt5a could also activate β-catenin signaling in a cultured cell line (called 293 cells) and found that it could not. But when they treated cells with both Wnt3a and Wnt5a, they discovered that Wnt5a could prevent activation of the β-catenin-dependent TCF transcription factor by Wnt3a. It was initially unclear how this happened. Wnt5a could compete with Wnt3a for the Frizzled receptor, or it might activate a gene that targets β-catenin for destruction. Either way, β-catenin



DOI: 10.1371/journal.pbio.0040131.g001

The Wnt5a protein can either activate or inhibit canonical Wnt signaling depending on which receptors are expressed. (CRD stands for the extracellular cysteine-rich Wnt binding domain present in the Fz4 and Ror2 receptors.)

levels should drop following treatment with Wnt5a. Yet β-catenin levels were unaffected; furthermore, Wnt5a didn't interfere with β-catenin's entry into the nucleus. These results indicate that Wnt5a did not block Wnt3a signaling through either of these routes. The researchers also show that Wnt5a doesn't rely on calcium-dependent signals, as had been suggested in previous work. Thus, Wnt5a must act through some other pathway to block β-catenin signaling by canonical Wnts such as Wnt3a.

Previous studies had suggested that Wnt5a might be able to bind another cell-surface receptor, Ror2, based on evidence that blocking expression of either Wnt5a or Ror2 produces the same effects in animals. And this line of investigation proved fruitful: Mikels and Nusse found that Ror2 is needed for Wnt5a-mediated repression of canonical β-catenin signaling. Additionally, by creating multiple Ror2 constructs lacking different combinations of their binding domains, they showed that Wnt5a binding triggers Ror2-mediated signaling inside the cell.

Interestingly, under very specific conditions—when the coreceptors Frizzled 4 (Frz4) and LRP5 are present—Wnt5a can actually trigger β-catenin accumulation and activate canonical β-catenin gene targets. Since 293 cells

do not normally express Frz4, but do express Ror2, the predominant signal prompted by Wnt5a in these cells is inhibition of β-catenin signaling—indicating that different combinations of cell-surface receptors drive different signaling outcomes for Wnt5a.

Whether Wnt5a inhibits β-catenin signaling—performing its job as a tumor suppressor—or activates β-catenin's cell growth and proliferation targets—setting the stage for tumor formation—depends on which receptors are present on the surface of the cell in question. The next challenge will be to identify the mechanism through which Wnt5a blocks the β-catenin pathway. By showing that one Wnt ligand can function through two separate pathways, Mikels and Nusse have opened the floodgates for researching the possibility of dual functionality in the 19 mammalian homologs identified so far. This ability to stimulate different receptors with distinct results is unique for Wnt proteins, but it has been documented in other systems and may well represent an alternate strategy for effecting flexible responses under changing conditions.

Mikels AJ, Nusse R (2006) Purified Wnt5a protein activates or inhibits β-catenin–TCF signaling depending on receptor context. DOI: 10.1371/journal.pbio.0040115

What Does Evolution Do with a Spare Set of Genes?

Mary Hoff | DOI: 10.1371/journal.pbio.0040132

A hundred million years ago, a molecular twist of fate endowed an ancestor of today's baker's yeast *(Saccharomyces cerevisiae)* with an extra copy of every gene it owned—the equivalent of a factory one day finding double the number of workers reporting for duty. What did the yeast and the forces of evolution do with this treasure trove of potential? Did the extra gene-workers simply double the output? Did the original crew and the duplicates divvy up the ancestral functions? Or did they take on new tasks? That's what Gavin Conant and Kenneth Wolfe sought to find out in their study of the networks of interactions among genes and other cellular components that emerged in the wake of that landmark event.

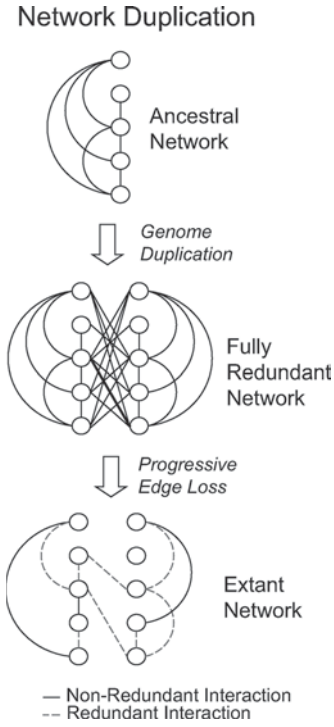
Some of the genes from the original doubling disappeared completely from the *S. cerevisiae* genome in the intervening millennia. But previous research had identified 551 duplicate gene (paralog) pairs that remain. To explore their fate, the authors used information about known co-expression from other *S. cerevisiae* studies along with an algorithm they developed on these genes pairs, and they identified 19 networks made up of paralogs divided such that there are many interactions within each network but few between the two paired networks. They then set out to explore the extent to which the networks composed of the two sets of paralogs differed from each other—a measure of the degree to which they had diverged evolutionarily, and so taken on separate functions, over time.

The first test looked at symmetry between the networks formed by the two sets of paralogs. The researchers found that for many of the network pairs, one set of paralogs had significantly more interactions than the other. The networks also had more redundancy—multiple interactions between two pairs of paralogs—than would be found in randomly grouped networks. These findings suggest substantial but incomplete divergence since the original gene-duplicating event.

Second, the authors explored the extent to which the 19 networks they had identified showed evidence of functional significance. To do so, they split the 551 paralog pairs into random networks, then recalculated network partitions for each. Eight of the networks showed significantly better clustering of gene interactions with respect to co-expression data than did the randomized networks, supporting the contention that they do in fact represent modular functional units, not just mathematical constructs. To further provide evidence of potential functionality, the researchers also analyzed whether partitions contained proteins with similar cellular localization and/or upstream regulatory sequence motifs. In the two largest of the networks with significant partitioning, protein localization and regulatory sequences were better conserved within each of the network partitions than would be expected by chance, confirming the functional correspondence seen with gene co-expression data.

To illustrate the adaptive value of network partitioning, the authors described a pair of paralogs whose protein products catalyze the last reaction in glycolysis. One encodes an enzyme induced by a compound present when glucose levels are high, while the other encodes an enzyme that works without this metabolic intermediate. As a result, the yeast can efficiently carry out the reaction in both high- and low-glucose environments.

Finally, the authors tested three mathematical models of network evolution against their observations as a way to gain insights into what actually happened to interactions



DOI: 10.1371/journal.pbio.0040132.g001

After a genome duplication event, which provides networks with many simultaneously duplicated genes (nodes), the number of nodes in the network has doubled and the number of interactions has quadrupled. The subsequent gain or loss of interactions reduces redundancy.

among genes over the evolutionary history of the yeast. In the first model, which they called "uniform loss," interactions were lost at random. In the second model, called the "poor-get-poorer" model, the probability of loss of an interaction between two genes was set to be inversely proportional to number of ancestral interactions retained. The third, "co-loss" model, in which the probability of an interaction loss depends on number of shared neighboring genes (the more shared, the less likely a loss) proved to provide the best approximation to which interactions actually were lost and retained over time. The strength of the third model supported the authors' speculation that the partitioned networks originally formed through the partial loss of old function rather than the development of new functions, in contrast to the common belief that increased complexity is largely the consequence of positive selection.

What *does* evolution do when handed a spare set of genes? In the case of *S. cerevisiae*, at least, it appears to have modified interactions among genes and other cellular components to produce a set of partially independent daughter networks from each single ancient network, creating in the process a division of labor that makes the most of the possibilities presented by the fortuitous duplication of the genome in the yeast's ancient past.

Conant GC, Wolfe KH (2006) Functional partitioning of yeast co-expression networks after genome duplication. DOI: 10.1371/journal.pbio.0040109

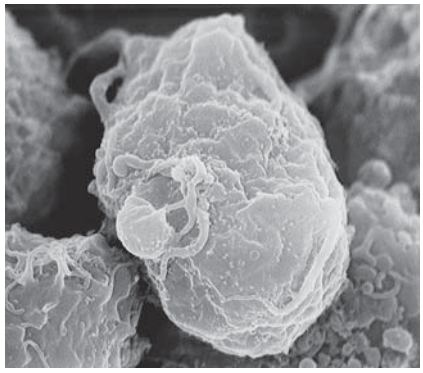
Inefficient Immune Killer Cells Abet HIV Infection

Liza Gross | DOI: 10.1371/journal.pbio.0040114

Viruses employ many strategies to elude their host's defenses, but it's tough to imagine a better tactic than targeting the immune system's communications director. HIV infects and kills helper T lymphocytes (also called CD4 T cells), the white blood cells responsible for mobilizing the mediators of an immune response. Infection results in high casualties among these cells, with half of the infected CD4 T cell population dying every 12 hours. It's thought that the response of another type of T cell—the cytotoxic T lymphocyte (CTL)—may be responsible for a large proportion of these deaths because CTLs recognize and kill infected cells. Based on this assumption, current HIV vaccine strategies are focusing on the CTL response. But it's unclear whether CTL activity or other mechanisms manage these killings, or how much ultimate control the CTL response exerts over the virus.

Evaluating the importance of the CTL response in patients with HIV has proven difficult, yielding conflicting results. In a new study, Becca Asquith, Angela McLean, and their colleagues turned to mathematical modeling to measure the overall effect of the CTL response on the lifespan of infected cells in patients with HIV. Applying different metrics to 28 independent datasets spanning 14 years, the researchers found that CTLs kill roughly 10 million infected cells a day—but, surprisingly, this represents just a fraction of infected cell deaths.

When a virus infects a cell, viral proteins get chopped up into short fragments called epitopes that bind to human leukocyte antigen (HLA) class I molecules and are presented on the infected cell surface. Viral epitopes bound to the HLA molecules act as a signal to the CTL immune response, informing it that the cell is infected and needs to be killed. Patients with HIV born with different forms of these HLA genes experience different rates of progression to AIDS. HIV can develop mutations in its epitopes that allow the virus to escape detection by CTL, often by abrogating HLA binding. The observation that these escape variants outgrow the wild type is much cited evidence that the CTL response exerts selective pressure on the virus.



DOI: 10.1371/journal.pbio.0040114.g001

The cytotoxic, "killer," T lymphocyte response kills only a fraction of the total number of HIV-infected lymphocytes in humans infected with the virus. The small spheres visible on the cell surface are viral particles. (Image: C. Goldsmith, CDC)

Asquith et al. have taken this argument one step further and quantified the rate at which escape variants grow out in order to estimate the CTL selective pressure. They developed a model of infected cell dynamics to quantify the rate of escape and fitness cost of escape mutations (since some mutations that confer escape may impair viral replication)—and, thus, the rate at which the CTL response kills virus-infected cells in vivo.

Using published data on 21 escape variants from 12 patients with HIV, the authors estimated each variant's rate of escape, and found "remarkably consistent" results: the rate of escape was very low—less than 0.1 day^{-1}—for 95% of the variants. (This means that it would take an escape variant about five months to outgrow and replace the wild-type population.) The researchers showed that this low escape rate did not occur because the escape variant carried a high fitness cost. They determined this by estimating viral reversion rates—the rate at which an escape variant reverts to wild type after infecting a patient lacking the selective HLA allele—in seven datasets collected from five patients. From these results, Asquith et al. calculated the average rate of escape and reversion for an escape variant to estimate the rate of cell death induced by a single CTL response. Just 2% of infected cell death could be assigned to CTL responses to a single epitope. With indications that a patient with HIV averages between 14 and 19 CTL responses, it's possible as many as 20% of infected cells are killed by CTLs. Thus, while CTLs play an important part in controlling infection, other factors mediate the majority of cell death.

This result helps explain the conflicting findings about the

importance of the CTL response in fighting infection—that specific HLA class I alleles are associated with delayed AIDS onset, yet infected cell death rates are not reduced in patients with advanced disease (which suggests that an impaired CTL response does not underlie disease progression). It may be that the modest contributions of the CTL response simply weren't detected in overall cell death rates. Indeed, using the authors' more sensitive approach of only quantifying infected cell death attributable to CTL (rather than total infected cell death), a significant weakening of the immune response with disease progression could clearly be seen. More importantly, these results suggest that unless vaccines based on the CTL pathway are formulated to be far more efficient than the natural response, they are not likely to prevent or clear HIV infection—but it's possible that a CTL-based vaccine might control the number of infected cells and help postpone the onset of symptoms.

Asquith B, Edwards CTT, Lipsitch M, McLean AR (2006) Inefficient cytotoxic T lymphocyte–mediated killing of HIV-1-infected cells in vivo. DOI: 10.1371/journal.pbio.0040090

A New Model for Predicting Outbreaks of West Nile Virus

Liza Gross | DOI: 10.1371/journal.pbio.0040101

Infectious diseases were wreaking widespread havoc long before scientists had any idea what caused them. But knowing the pathogenic agents behind today's scourges is just the first step in protecting against deadly outbreaks. Roughly 75% of emerging infectious diseases are zoonotic—humans contract them either directly from infected animals or through vectors that feed on infected animals. West Nile virus is the biggest threat in North America, where *Culex* mosquitoes are the primary vector. Birds are their main target, but mosquitoes also transmit the virus to humans, horses, and other mammals.

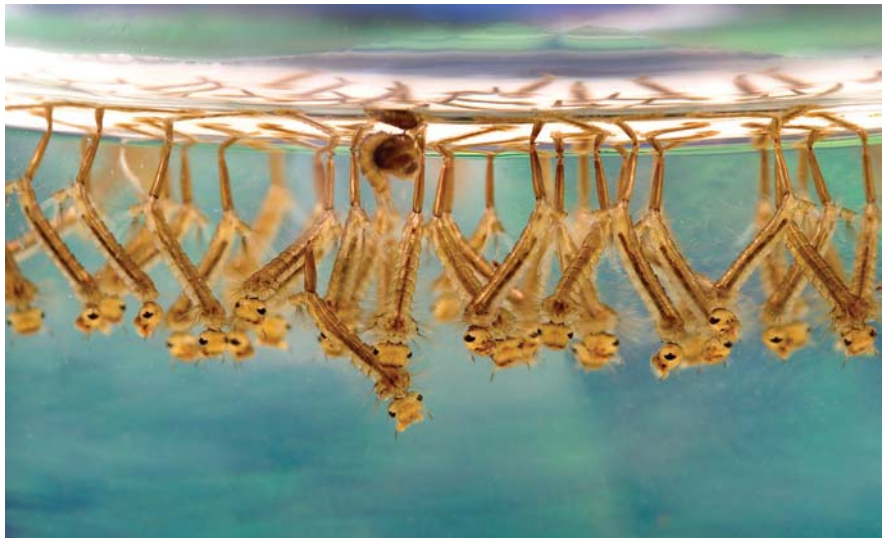
Since the virus was first discovered in New York City in 1999, it has infected 20,000 people and killed 770—in stark contrast to the sporadic infections in Europe. The factors behind the North American epidemics are poorly understood, though proposed explanations involve a more virulent strain, North American birds' ineffectual immune response, and a hybrid species of mosquito that prefers humans over birds. In a new study, A. Marm Kilpatrick, Peter Daszak, and their colleagues now present evidence that a shift in *Culex pipiens* mosquito feeding behavior from birds to mammals is also driving the epidemics.

A critical factor in predicting the intensity of a zoonotic epidemic involves determining how the vector's feeding behavior and preferences change over space and time. Birds appear to be West Nile's most competent vertebrate host—they transmit the virus to other mosquitoes, which supports viral reproduction—while humans (and most other mammals) can't transmit the virus. The researchers hypothesized that if mosquitoes bit mostly birds in the summer, then switched to humans in the fall, this behavior could intensify both the summer epidemic in mosquitoes and the subsequent transmission to humans.

To investigate this possibility, Kilpatrick et al. collected data from six sites in Maryland and Washington, D. C., from May through September 2004, to determine the population dynamics of birds and mosquitoes, which taxa *Culex* was targeting, and the epidemiology of the virus. They estimated population densities for mosquitoes and birds at each site, and identified the morphologically cryptic mosquitoes by sequencing their DNA. Over 90% of their catches were *Cx. pipiens*, which were tested for the virus. The researchers determined species of avian and mammalian targets by sequencing the DNA from blood in engorged mosquitoes.

From May to June, the American robin, which represents just 4.5% of the local avian species, accounted for over half of *Cx. pipiens*' meals. As the summer wore on, and robins left their breeding grounds, the probability that humans would provide the blood meal increased 7-fold, while the probability that *Cx. pipiens* would feed on robins declined. Since the birth of new offspring raised the overall numbers of birds during this same period, Kilpatrick et al. concluded that mosquitoes switched to humans when robins—their preferred host—dispersed.

With the data collected from the Washington, D. C., area, the researchers modeled the risk of *Cx. pipiens*–mediated viral transmission to humans based on *Culex* mosquito abundance, the prevalence of *Culex*



DOI: 10.1371/journal.pbio.0040101.g001

A shift in the feeding behavior of *Culex* mosquitoes (their larvae amass in standing water, as seen above) helps explain the rising incidence of West Nile virus in North America. (Image: James Gathany, CDC)

infection, and the probability that mosquitoes would feed on humans. The model predicted that the risk of human infection peaked in late July to mid-August, declined toward the end of August, then rose slightly at the end of September. The pattern of actual human cases in the area, the authors point out, "showed a strikingly similar pattern." The model also suggests that the human incidence of West Nile virus would have been much lower if mosquitoes had maintained their June feeding rate throughout the season.

The same pattern was seen in California and Colorado, with a peak abundance of infected *Cx. tarsalis* mosquitoes in June and July, followed by a late-summer spike in human infections. Since mosquitoes feed primarily on birds during early summer, viral load can increase substantially. When mosquitoes switch to humans, the prevalence of infection among mosquitoes increases the chances of a human epidemic. If mosquitoes had fed mostly on humans—wasted meals from the perspective of viral amplification—instead of birds during early summer, prevalence of infection in mosquitoes and then humans would have been greatly reduced.

These feeding shifts appear to be a "continent-wide phenomenon," the researchers conclude, and may also explain outbreaks of other avian zoonotic viruses. This study highlights the importance of understanding how vector behavior affects transmission of zoonotic pathogens to humans—a crucial step in developing strategies to prevent and control a potential epidemic.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P (2006) West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. DOI: 10.1371/journal.pbio.0040082

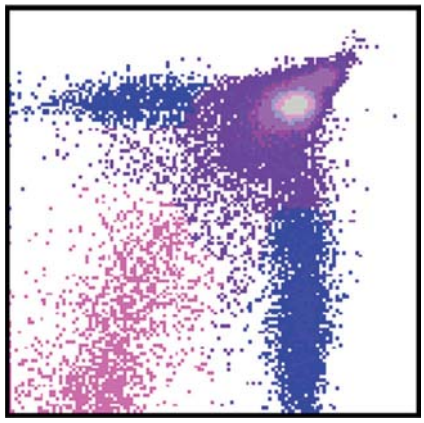
The Education of Mr. T

Caitlin Sedwick | DOI: 10.1371/journal.pbio.0040117

T "helper" cells (which express the surface marker CD4) and "killer" T cells (which express CD8 markers) are each critical for detecting and neutralizing microbial invaders and protecting the body from disease. Both types of T cells recognize foreign invaders through surface expression of a T cell receptor (TCR) that is unique to each T cell. When an infected cell expresses protein fragments (peptides) derived from a pathogen on its surface, it raises a red flag for the TCR that recognizes the peptide.

Before CD4 T helper cells or CD8 killer T cells can be unleashed on invading armies of microbes, they must first learn how to detect appropriate targets for their activities. This education process takes place in the thymus (thus, the "T" in their name), where T cells originate. Immature cells that will eventually become T cells come to the thymus from the bone marrow. Once they arrive in the thymus, immature T cells (now called "thymocytes") undergo specific maturation steps that result in the simultaneous surface expression of both CD8 and CD4 proteins.

Later, they will choose to express only one of these determinants, but these "double-positive" thymocytes must first pass two sequential life-and-death tests. First, they undergo positive selection to make sure they have a functional TCR. Then they undergo negative selection to ensure that their TCR does not strongly recognize determinants derived from body proteins (self). If a prospective T cell expresses a defunct TCR on its surface, it will fail the positive selection test and undergo "death by neglect." Then, if it expresses a TCR that responds too enthusiastically, it will fail the negative selection test and will also die. When T cell education goes awry, an individual faces dire consequences, including severe immune deficiency or autoimmune disease.



DOI: 10.1371/journal.pbio.0040117.g001

A representative flow cytometry profile of CD4 and CD8 expression in adult mouse thymocytes. Researchers can use such data to track the progression of thymocytes through different developmental stages.

In both positive and negative selection, T cell death is accomplished by a process called apoptosis (cellular suicide), in which the Bcl-2 family of proteins plays a much-celebrated role: some Bcl-2 family members (including Bcl-2 and Bcl-xL) protect cells against apoptosis, while others (such as Bax, Bak, and Bid) promote it. Scientists still struggle to understand how the activity of Bcl-2 proteins is regulated inside cells.

In an effort to identify other proteins involved in the education of T cells, Takeshi Nitta, Yousuke Takahama, and their colleagues undertook a screen for genes whose expression changes in T cells during positive selection. The screen identified the immune-associated nucleotide-binding (IAN; also known as GIMAP) family of proteins, which are all expressed in immune tissues, as players in this process. In particular, the researchers identified *IAN1*, *IAN4*, and *IAN5* as participating in the process of T cell education, through an interaction with various Bcl-2 family members.

Nitta and his colleagues first characterized the expression pattern of the IAN family generally: in the mouse, there are eight IAN family proteins (IAN1–7 and IAN9), whose coding genes are all packed together on Chromosome 6. As expected, they found the genes of these proteins expressed predominantly in immune tissues (thymus, spleen, lymph nodes, and bone marrow) and also in the lung. When they looked for expression of these proteins in T cells, they found that expression of *IAN1*, *IAN4*, and *IAN5* increased in thymocytes during the process of positive selection.

To further probe the functions of these proteins, the authors engineered thymocytes to prematurely

overexpress either *IAN1*, *IAN4*, or *IAN5*. Under these conditions, cells overexpressing *IAN1* died prematurely (at the CD4, CD8 double-positive stage), while those expressing *IAN4* or *IAN5* were not affected. Conversely, when Nitta and his collaborators prevented thymocytes from expressing these proteins, they found that loss of IAN4 expression blocked positive selection, while loss of IAN5 prevented thymocytes from reaching the double-positive stage; loss of IAN1 expression had no effect. Subsequently, the authors found that IAN4 and IAN5 each associate with Bcl-2 proteins (of both the pro- and anti-apoptotic flavors), while IAN1 was found to associate specifically with Bax; the intracellular location of the individual IAN proteins also co-localized with their respective Bcl-2 binding partners.

Because the expression of these three IAN proteins is carefully timed during T cell development—and because they interact with Bcl-2 family members and appear to regulate T cell survival—further investigation is warranted to determine the precise contribution of IAN proteins to the T cell developmental and survival process. There are also several questions remaining to be examined, including the structural basis of the IAN: Bcl-2 family protein association. The identification of IAN proteins' involvement in T cell development opens a rich trove of potential investigations.

Nitta T, Nasreen M, Seike T, Goji A, Ohigashi I, et al. (2006) IAN family critically regulates survival and development of T lymphocytes. DOI: 10.1371/journal.pbio.0040103

Remnants of the Past or Ready to Move? Resident Birds Display Migratory Restlessness

Liza Gross | DOI: 10.1371/journal.pbio.0040130

In a remarkable display of endurance and fitness, arctic terns fly up to 20,000 miles between their Arctic breeding grounds to the Antarctic seas each year. But most long-distance fliers rack up considerably less mileage, and rely on extra fat storage rather than snacking along the way, as terns do. Still other migrating birds travel just a few miles between alpine meadows and lowlands to find optimal food and shelter. Some fly at night, others during the day; some over land, others over water. No one can say for sure how migration came about, but climate, competition for resources, and the availability of food all likely played some role in this ancient behavior.

Studies of migratory behavior have shown that captive migratory birds demonstrate a seasonally appropriate spontaneous urge to migrate, called Zugunruhe (pronounced zook-oon-roo-ha). This behavior varies with the species studied, with amount and direction of activity reflecting the species' natural migratory distance and route, suggesting that the migratory urge is innate. In a new study, Barbara Helm and Eberhard Gwinner took a different approach to studying migratory behavior. Rather than focusing on a migrating species, they decided to investigate the possibility that resident species also bear elements of Zugunruhe—and discover that "a readiness to move is common in birds."

Helm and Gwinner searched for signs of migratory behavior in two subspecies of stonechats, *Saxicola torquata*, comparing a migrant that breeds in Austria, *S. t. rubicola*, and its equatorial resident relative, *S. t. axillaris*. European stonechats are short-distance, nocturnal migrators—they winter around the Mediterranean Sea—that begin their journey when daylight lasts just over 12 hours. Since they would otherwise be sleeping at night, nocturnal activity can serve as a proxy for Zugunruhe. African stonechats are sedentary species that do not abandon their breeding grounds in Kenya. Since the genetic and evolutionary divergence between stonechat taxa is large (these two subspecies diverged between 1 million and 3 million years ago), it's reasonable to predict that African stonechats would neither possess an internal migratory program nor display migratory restlessness. On the other hand, the evidence that migratory birds adjust their flight patterns in response to environmental changes and the suggestive evidence that resident birds display traces of migratory restlessness raises the possibility that migration may not be an all-or-nothing trait.

DOI: 10.1371/journal.pbio.0040130.g001

Recording migratory restlessness in resident African stonechats (the European stonechat is pictured above) suggests that nonmigratory birds retain an innate program supporting a seasonal urge to migrate.

To investigate the presence of Zugunruhe in a resident species, the researchers raised and bred the offspring of Kenyan stonechats in their lab in Germany. One group of these birds was held for the duration of a migratory period under the nearly equal light and dark conditions of their native habitat, and a subset remained under these conditions for a year and a half. A control group was exposed to the natural seasonal light fluctuations of southern Germany. Helm and Gwinner recorded the birds' nocturnal movements with infrared motion sensors, and counted the number of movements within ten-minute intervals. If 20 or more movements were noted, the interval was considered "active."

Even though the African stonechats experienced no temporal cues—light levels remained constant—their nocturnal activity roughly tracked the season. The African birds' migratory restlessness, marked by repeated, spontaneous outbursts of nocturnal activity, echoed that seen in European stonechats, though it was less pronounced. The African birds also showed a telling relationship between hatching date and onset of nocturnal activity: just like their migratory counterparts, late-hatching birds became restless earlier and earlier, coinciding with the migratory season.

The African birds' behavior can be attributed only to Zugunruhe, the

researchers concluded, suggesting the influence of an inborn, precisely timed migratory program. The presence of this program in both migrants and residents suggests that the urge to migrate may have evolved in their common ancestor.

It's not clear what mechanism preserved the trait in the residents. It could be adaptive: Southern African stonechats, it's thought, migrate short distances up and down mountains, so it's possible that drought or other seasonal conditions could force the Kenyan birds to periodically take wing as well. Alternately, stabilizing selection may have protected the trait from extreme variation, or it may support the dispersal of young birds to new territories.

Whatever forces have retained this trait, Helm and Gwinner propose that it may be a common avian feature. Given the proper environmental triggers, this innate migratory program might kick in to allow birds to escape deteriorating habitats caused by global climate changes or other ecological disturbances. With evidence that Zugunruhe exists in nonmigratory birds, researchers can continue exploring migratory behavior in any number of resident-migratory pairs to probe the many ways birds take flight to improve their chances of survival.

Helm B, Gwinner E (2006) Migratory restlessness in an equatorial nonmigratory bird. DOI: 10.1371/journal.pbio.0040110

Intensive Training Reveals Plasticity in the Adult Auditory System

Liza Gross | DOI: 10.1371/journal.pbio.0040104

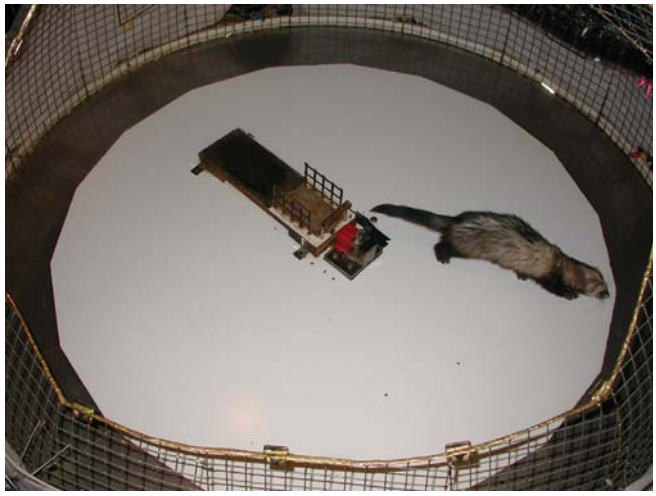
When your exuberant kitten races too far up a tree to negotiate a safe descent, you can quickly locate your distressed charge by homing in on her plaintive cries. In the wild, mammals from Norway rats to elephant seals rely on sound localization skills to escape threats, forage, and communicate. The brain networks underlying these skills are largely shaped by experience during development. Though the adult brain retains some capacity for rewiring these networks, it's not clear which tasks benefit from plasticity or how the brain engenders this flexibility.

In a new study, Oliver Kacelnik, Andrew King, and colleagues investigated these questions by manipulating the hearing of adult ferrets. They show that ferrets with obstructed hearing (an earplug in the left ear) can quickly adapt to altered auditory cues when trained to do so in the service of a meaningful task (getting a drink). Frequent training was crucial in boosting the rate and extent of their improvement.

To localize sound, the brain processes spatial cues in the sound waves that enter each ear. In interpreting sounds on the horizontal plane (imagine a coyote approaching from the side), the brain uses disparities in how sounds reach each ear, called interaural time differences and interaural level differences. Changes in level at different frequencies (the sound's spectral properties) are produced by the external ear and can reveal the source's elevation (think swooping raptor), whether it's coming from front or back, and sometimes help with monaural localization.

To study adaptive hearing in an adult mammal, the authors blocked the left ears of ferrets with specially outfitted earplugs and measured their ability to localize sounds in the horizontal plane. To do this, the authors trained ferrets to approach a sound source to get a water reward. The ferrets licked a waterspout, triggering a burst of noise from one of twelve loudspeakers. Ferrets that approached the correct speaker received the reward. Performance was based on accuracy—did they approach the sound source?—and on head-orienting responses (which were compared with unconditioned, pre-plug head-orienting behavior).

Ferrets were presented with sound bursts lasting either 1,000 or 40 milliseconds. Without earplugs, ferrets had no trouble localizing the 1,000-millisecond sounds; they were slightly less adept at localizing the brief bursts. With earplugs, performance dropped considerably for both bursts. Ferrets had the most trouble with sounds coming from their left



DOI: 10.1371/journal.pbio.0040104.g001

Measuring the accuracy with which ferrets can localize sound.

(obstructed) side, but errors increased significantly for all 12 sound sources.

The authors retested one group of earplugged animals every six days to see if they could use the altered cues to recover their localization skills. By three weeks, the trained animals had shown a marked improvement, localizing sounds coming from the right (unobstructed) side about as well as they had before the ear was plugged. The speed and accuracy with which ferrets recovered their localization abilities depended on training, with more complete recovery occurring when the animals received more frequent training.

There is some evidence to suggest that visual cues might help to boost auditory plasticity. To test this possibility, the authors compared the earplugs' behavioral effects on two groups of ferrets: one blind since infancy, and another with normal vision. Both groups could localize about the same without earplugs, made more errors after receiving the plug, and then steadily improved with the periodic retesting. These results show that the ferrets could not only be trained to reprocess abnormal localization cues but also that they could do so without visual cues. Then, by training another group of ferrets to localize both auditory and visual stimuli before inserting the earplug, the authors show that sound

localization depends "exclusively on auditory training" and does not involve "a visual recalibration of auditory space." Furthermore, adaptation did not depend on error feedback, since rewards were not based on performance.

After removal of the earplugs, ferrets made errors reflecting a small bias toward the previously plugged ear: they initially processed the cues as if the ear was still plugged. This aftereffect, though transient, indicates that the adaptive response relies in part on reinterpreting the relationship between binaural cues and location. However, compensation for the earplug-disrupted binaural cues mainly involved the animals' learning to make use of other cues that were less distorted by the earplug, including low-frequency interaural time differences, and spectral cues provided by the unobstructed ear.

Altogether, these results show that the adult auditory system can adapt to abnormal spatial cues and can do so rapidly with intensive training. By recording brain activity as animals perform the tasks described here, future studies can shed light on the brain regions responsible for this plasticity. Whatever the mechanism, the finding that plasticity follows targeted, intensive training suggests that patients with hearing disorders might benefit from a similar strategy—providing more evidence that an old brain can sometimes learn new tricks.

Kacelnik O, Nodal FR, Parsons CH, King AJ (2006) Training-induced plasticity of auditory localization in adult mammals. DOI: 10.1371/journal.pbio.0040071

Tuning in to How Neurons Distinguish between Stimuli

Mason Inman | DOI: 10.1371/journal.pbio.0040118

Most anyone can tell grape juice from fine wine with a quick sip, but distinguishing a French Syrah from an Australian Shiraz requires a more refined palette, plus perhaps a bout of sniffing and swirling. Similarly, our sensory neurons' ability to make distinctions depends on the task at hand: in some cases, they excel at making coarse distinctions; in others, they are better at hairsplitting.

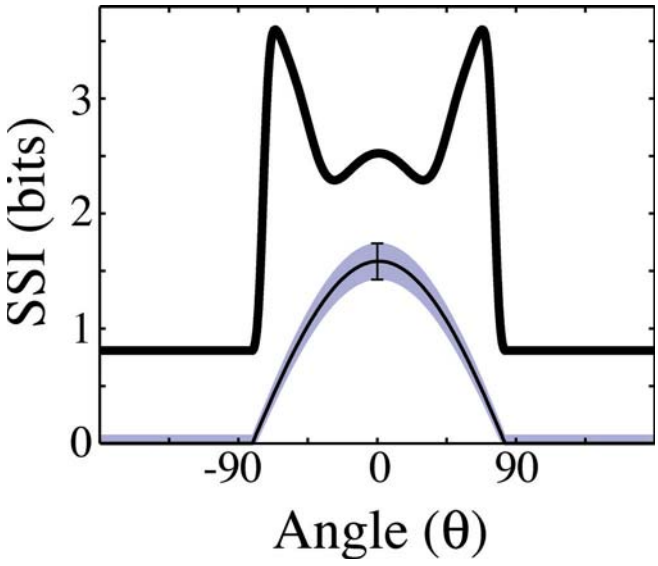
Finding out what exactly particular neurons are responding to is not as easy as asking a fellow diner which wine they prefer. But using a mathematical measure of information, a new study by Daniel Butts of Harvard University and Mark Goldman of Wellesley College reveals when specific cells are best at discriminating among different stimuli.

Listening in on neuronal activity, neuroscientists can see that a field of vertical stripes flashed in front of a cat's eyes, for example, will goad a certain set of neurons into firing frenetically. Similarly, horizontal stripes will set another set of neurons firing, and diagonal stripes will activate all these neurons at some intermediate level.

So tweaking the stimuli—say, rotating the stripes—shows how a particular neuron or set of neurons responds to various stimuli, and the set of results can be represented with a so-called tuning curve. These curves are typically bell-shaped with a peak around one point where neurons are firing most quickly—corresponding, say, to stripes held vertically—and drop off to low activity on either side.

However, it hasn't been clear which part of the tuning curve is most significant: does the peak reflect the stimuli a neuron is best at detecting? Or are the steep slopes of the curve most important, since this is where a small change in the stimulus elicits the greatest change in the neuron's response? Studies of actual and simulated neuronal activity have given mixed results, without an overarching theory to explain them all.

In the new study, Butts and Goldman applied a mathematical measure of information, which they call the stimulus-specific information (SSI), to neurons' tuning curves. If a neuron has a very reliable response to a certain stimulus, it is straightforward to spot this response. But if the neuron fires more sporadically, it is harder to say for sure if the neuron is responding to that stimulus or not. By calculating the SSI for neurons' responses, Butts and Goldman were able to quantify how this uncertainty affected the ability of a neuron to convey information about various stimuli.



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This "Batman" graph shows which stimuli are best encoded by a neuron in a relatively noise-free situation, when the neuron is better at making fine distinctions.

The analysis reveals that this noise in the neurons' response is crucial to figuring out when neurons are most discriminating. In general, when the neurons' responses were less noisy, the neurons were best at fine discrimination. In this case, a graph of the SSI could resemble a silhouette of Batman's head: two sharp peaks, corresponding to the slopes of the tuning curve, with a smaller bump in the middle, matching the tuning curve's peak. When the neurons' response was noisy, however, the neurons were making more coarse distinction, and the graph of the SSI mirrored the tuning curve, showing a single peak where the neuron was firing most rapidly.

With their measure of SSI, Butts and Goldman have shown a way to connect neurons' responses with how they process information and signal their neighbors. So just because a neuron is firing lackadaisically, that doesn't mean it's not getting the job done.

Butts DA, Goldman MS (2006) Tuning curves, neuronal variability, and sensory coding. DOI: 10.1371/journal.pbio.0040092

What Governs Enzyme Activity? For One Enzyme, Charge Contributes Only Weakly

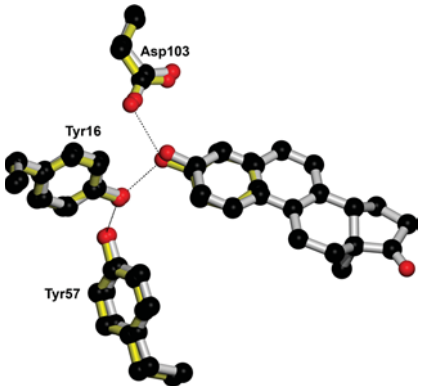
Richard Robinson | DOI: 10.1371/journal.pbio.0040133

The sugar on your table and the oxygen in the air don't spontaneously ignite, but why not? The answer is that the conversion from reactants—sugar plus oxygen—to products—carbon dioxide plus water—requires the reactants to first adopt an extremely unstable configuration, called the transition state, in which their bonds are weakened, but newer, stronger ones have not yet formed. The "energy hill" that separates reactants from the transition state is just too high, so your sugar remains stable at room temperature.

Not so inside a cell, where enzymes catalyze thousands of different reactions that would take days, or millennia, without them. There, a reactant—called a substrate—fits into the enzyme's active site, a pocket or groove on its surface. The active site is lined with chemical groups whose shape and charge complement the shape and charge of the substrate, positive meeting negative, bump nestling into hole.

But while the reactant fits in nicely, much of the catalytic power of the enzyme has been thought to be derived from making an even better fit with the transition state. To do this, the enzyme first forms weak, temporary bonds with the reactant. The shape and charge of the active site are such that, as the reactant deforms into the transition-state configuration, those bonds become stronger. Thus, the enzyme can stabilize the transition state, lowering the height of the energy hill and thereby increasing the probability that the reactants will convert into products. Enzymes typically speed up a reaction by many orders of magnitude—a rate increase of a trillion-fold is routine for enzymes.

Shape and charge complementarity between enzyme and substrate have been proposed as keys to enzyme function, but are both equally important? That question is devilishly hard to answer, for the most fundamental of reasons: shape and charge are interdependent in most cases, and altering a molecule's shape (by inserting a larger atom, say) also changes its charge distribution. In a new study, Daniel Kraut, Daniel Herschlag, and colleagues separate the two effects and show that, for at least this one enzyme, charge makes only a modest contribution to catalytic power.



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Superimposition of complexes formed between the active site of ketosteroid isomerase and two transition-state analogs.

The enzyme ketosteroid isomerase (KSI) rearranges the bonds within its substrate, a multi-ring steroid molecule, by shifting a hydrogen ion from one carbon to another. One step in this process is the formation of two weak, temporary bonds, called hydrogen bonds, between KSI and an oxygen atom on the substrate. As the substrate deforms into the transition state, this oxygen becomes partially negatively charged, and the hydrogen bonds become stronger.

KSI can bind other molecules that fit the active site, including one called a phenolate anion. This compound has an oxygen atom in the same position as the steroid oxygen, but phenolate's oxygen is negatively charged, mimicking the transition state for the steroid. That charge can be made weaker or stronger by adding different chemical groups to the far end of the phenolate. Because these additions are made away from the active site, the shape of the molecule within the active site doesn't change, and the authors could evaluate charge independent of shape.

The authors did not measure reaction rate directly, but instead measured a key factor that determines reaction rate, the strength of binding interactions formed to the variably charged phenolate anion—a simple-enough sounding procedure that nonetheless drew on the full range of tools in the modern chemist's toolbox, from NMR spectroscopy to calorimetry to X-ray crystallography. Over the entire range of compounds tested, they found a difference in binding strength of only 1.5-fold, corresponding to an estimated change of at most 300-fold in the reaction rate. The authors propose that several other factors, including shape, each contribute modestly to catalysis.

While these results are directly applicable to only KSI, they provide a window onto the factors affecting catalysis in many other enzymes. Calculations based on these results may allow estimation of the effects of charge in other enzymes that cannot be manipulated in this same way. The complementary experiment—altering shape while keeping charge constant—may be even harder, and remains to be done.

Kraut DA, Sigala PA, Pybus B, Liu CW, Ringe D, et al. (2006) Testing electrostatic complementarity in enzyme catalysis: Hydrogen bonding in the ketosteroid isomerase oxyanion hole. DOI: 10.1371/journal.pbio.0040099