Bridging the Gap between Theory and Ecology in Evolutionary Models

Liza Gross | DOI: 10.1371/journal.pbio.0040405

The history of life is filled with examples of one species diverging into several, even thousands, each with unique traits geared to the demands of its ecological niche. In the textbook case of adaptive radiation, an ancestral finch species landed on the Galapagos Islands just a few million years ago, and evolved into 13 new species with specialized beaks adapted to exploiting the various seeds, nuts, insects, and other food sources on the island.

Adaptive radiations suggest that species evolution follows the first rule of business: find a niche and fill it. But that's not what most models used to detect evolutionary patterns of trait evolution assume. And in a new study, Robert Freckleton and Paul Harvey demonstrate the limitations of that choice. They also introduce a method to minimize those limitations by using a diagnostic tool that can detect evolutionary patterns that deviate from the standard models.

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Old World Leaf warblers and Dendroica warblers (*Dendroica fusca*, pictured above), two classic cases of adaptive evolution, served as case studies for two diagnostic tests designed to reveal deviations from a Brownian motion model of trait evolution.

The complexity of evolutionary processes and spottiness of the fossil record calls for statistical models—whose accuracy depends on their assumptions—to infer historical patterns of evolution. Traditional approaches to studying the evolution of traits (such as beak shape) typically compare populations, species, or higher taxa to identify adaptations and the corresponding evolutionary processes. With advances in molecular genomic techniques, comparative methods increasingly incorporate phylogenetic analyses, which compare gene or protein sequences to infer evolutionary relationships between taxa or traits.

These phylogenetic comparative methods often use a "Brownian motion" model of evolution, which assumes that more closely related species are more similar to each other and generate expected distributions of trait change among the species compared. Freckleton and Harvey suspected that the models could produce specious correlations, because they don't explicitly account for ecological processes. Such a model—which, the authors point out, has rarely been tested—assumes (among other things) that traits evolve at a constant rate over time.

Freckleton and Harvey analyzed real and simulated data using a niche-filling model and a Brownian motion model and then applied two statistical tests as diagnostic tools to detect patterns of trait evolution that fall outside the assumptions of the Brownian motion. In the niche-filling model, niche space is initially empty (much like Darwin's finches may have encountered), and new niches arise at a given rate, in random positions, and are instantly invaded by species with traits suited to exploiting that niche. Evolution occurs only when a new niche—such as a novel seed—appears and a species is under selection to exploit it. In contrast to Brownian models, for example, one would expect that as niches became filled with more species, the difference between the parent and offspring species would become smaller, because niches have a unique optimum value and trait values are constrained (by correlations between beak size and food size, for example). Likewise, with an adaptive radiation, one would expect ecological differences to arise with or shortly after speciation, rather than at a constant pace dependent only on time.

Speciation rate—defined as the rate at which new niches appear and are invaded in the niche-filling models and the rate at which lineages split in Brownian motion models—was modeled using three different models: the probability of speciation is proportional to the number of species present, remains constant, or declines with the number present. Each scenario reflects a different process corresponding to the invasion of an empty niche.

The two diagnostic tests included a "node height" test, which assesses whether the rate of evolution of a particular trait occurs systematically within a phylogenetic tree, and a simple randomization test. (Node height refers to the distance between the ancestral species, or root, and the most recent common ancestor for a pair under study.) In the latter case, if the data are consistent with Brownian motion, one would expect small and large changes of a particular trait (such

as beak size) to be equally likely at any point in the phylogenetic history of the group of species compared .

The authors first used simulated data to provide statistical confidence levels for their two tests and showed that the power of each test to detect non-Brownian evolution depended on the model of speciation as well as the extent of correlation between traits. They then applied the tests to published data on the phylogeny and feeding habits of two warblers, both classic cases of adaptive radiation. Both statistical tests were able to detect non-Brownian evolution of two feeding-related traits (body size and prey size) in Old World Leaf warblers. In a second case, neither test detected deviations from the Brownian model for the evolution of beak shape and size in Dendroica warblers—indicating that Brownian motion correctly described the pattern of trait evolution in this case, which provided a case study for the alternative scenario.

The authors emphasize the diagnostic nature of these tests and the need for developing more-refined techniques to detect deviations from Brownian evolution. But their results underscore the importance of incorporating ecological processes into comparative models, to provide a more realistic and detailed account of the historical pressures and mechanisms driving the diversification of life.

Freckleton RP, Harvey PH (2006) Detecting non-Brownian trait evolution in adaptive radiations. DOI: 10.1371/journal.pbio.0040373

Can DNA Distortion Turn RAG into a Potent Transposase?

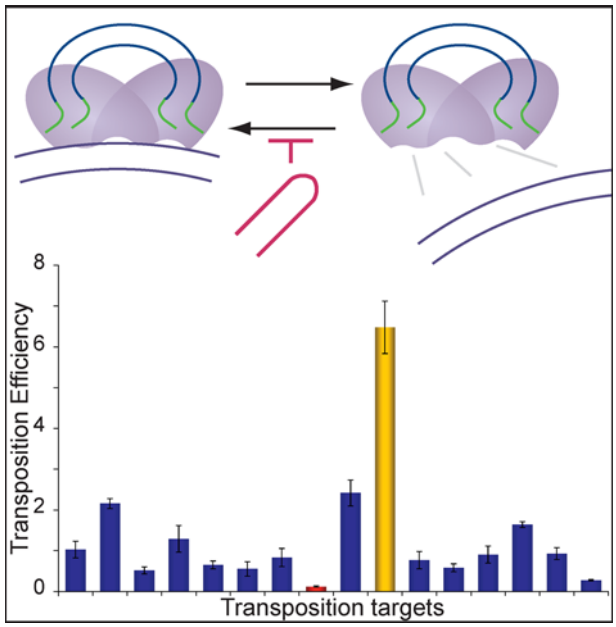
Liza Gross | DOI: 10.1371/journal.pbio.0040390

As a general rule, DNA rearrangements spell trouble. By facilitating the movement of genetic elements to new sites in the genome, one class of transposition enzymes—the transposase/retroviral integrase superfamily—plays a major role in disease. Transposases can cause cancer by reinserting DNA into or near cancer-related genes. Retroviral integrases pave the way for HIV infection by integrating the retrovirus into the genome. But genetic rearrangements, mediated by a recombinase produced by the recombination activating genes *(RAG)*, also underlie the body's ability to ward off infection. By recognizing specific bits of DNA called recombination signal sequences (RSS) that bookend DNA separating two gene fragments, RAG complexes can remove the intervening DNA and join the two gene fragments remaining in the immune cell receptor gene locus. This genetic reshuffling process, called V(D)J recombination, generates the phenomenal diversity of immune cell antigen receptors that can recognize virtually any pathogen that slips into the body.

In the late 1990s, researchers discovered that the RAG complex can also act like a transposase, by reinserting DNA segments into unrelated DNA targets. This suggested that RAG-mediated transposition might trigger the chromosomal translocations seen in lymphoid tumors. But since RAG-mediated transposition was found only in "cell-free" test tube experiments, not in living cells, it was thought that cells pulled out the regulatory stops to inhibit RAG transposition and protect genomic stability.

In a new study, Jennifer Posey, David Roth, and colleagues show that RAG can mediate transposition quite effectively—provided the right target is available. Their findings could explain why researchers have had such a hard time finding evidence of RAG transposition in living cells.

Because transposases often exhibit clear biases for certain DNA targets, Posey et al. suspected that target-site selectivity might provide the regulatory means to block RAG transposition without preventing its V(D)J recombination activity. Early studies suggested that RAG transposition preferentially targets stretches of DNA rich in guanine (G) and cytosine (C) nucleotides, especially certain GC hotspots. But more recent evidence indicates that RAG transposition favors distorted DNA structures called hairpins—single-stranded DNA that folds back on itself to form a loop—at the tips of a "stem" of nucleotides. (When this "stem and loop" structure forms on both strands of DNA, it is called a cruciform.)



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RAG transposition, thought to be rare, is actually robustly stimulated by the correct hairpin targets. One structure, however, inhibits transposition by preventing target capture.

Because the last four nucleotides of a hairpin provide targets for other DNA-cleaving enzymes (called endonucleases), the authors thought the terminal ends of hairpins might do the same for RAG transposition. To investigate this possibility, they generated a set of 16 DNA fragments, covering all possible four-nucleotide combinations around the hairpin tip, each having the same stem and a different hairpin tip. They incubated each tip with RAG proteins and RSS-bounded DNA segments and calculated transposition efficiency as the percentage of RSS ends transposed into the hairpin target.

Transposition efficiency ranged from "virtually undetectable" to "robust," depending on the tip's nucleotide

sequence. Still, most of the hairpins acted as strong targets. Interestingly, GC tips generated far more activity than CG, indicating that transposition depends on more than nucleotide content alone. Rather, the sequence of the four nucleotides around the hairpin determines the structure of the tip and thus how attractive a target it will be for RAG transposition. When the nucleotide sequences support a cruciform structure, they stimulate the most efficient transposition.

The exception to the rule is the CT (cytosine-thymine) hairpin, which actually inhibited transposition, even though it did not inhibit the RAG proteins' ability to cleave DNA and could bind to the RAG/RSS complex. Interestingly, a CT sequence that did not adopt a cruciform structure had no inhibitory effect on transposition. It may be that the CT hairpin interferes with RAG activity by somehow preventing the RAG complex from successfully capturing the target—a possibility that can be explored in future experiments.

By showing in the test tube that the RAG complex can readily stimulate transposition when it encounters a preferred target, this study should stimulate new searches for RAG transposition in living cells. Given the RAG proteins' highly specific target preferences, it's not surprising that RAG transposition has been so hard to find in living cells. But now that researchers have a clearer idea of what to look for, they can look for the telltale signs of RAG transposition in lymphoid tumors to shed light on its potential contributions to cancer.

Posey JE, Pytlos MJ, Sinden RR, Roth DB (2006) Target DNA structure plays a critical role in RAG transposition. DOI: 10.1371/journal.pbio.0040350

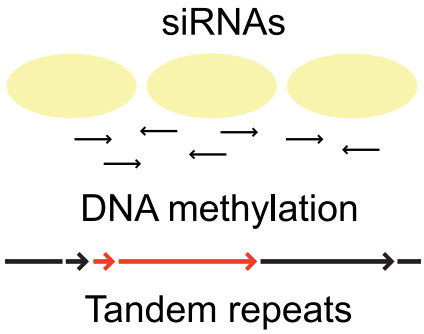
siRNAs and DNA Methylation Do a Two-Step to Silence Tandem Sequences

Richard Robinson | DOI: 10.1371/journal.pbio.0040407

The genomes of higher organisms, including plants, are riddled with repetitive sequences, remnants of self-copying DNA parasites that randomly reinsert themselves, often harmlessly, but occasionally disrupting genes. Silencing these repeated elements is a major challenge for maintaining genomic health and is a major function of DNA methylation. In this process, a CH3 group is added onto one of the four DNA bases; groups of these altered bases reduce RNA polymerase's access to the DNA, preventing transcription.

One common repeated element seen in genomes is tandem repeats, pairs of identical short DNA sequences lying next to each other. A longstanding question is how methylation machinery is directed to these tandem repeat sequences, which are frequently transcriptionally silenced. In a new study, Simon Chan, Steven Jacobsen, and colleagues show that both members of the pair are required, and their presence first stimulates production of small interfering RNAs (siRNAs). The siRNAs then attracts DNA methyltransferase, the enzyme directly responsible for methylation.

Only recently discovered, siRNAs have begun to pop up in many gene regulatory events. First transcribed as a larger RNA molecule, then diced into small fragments, siRNAs appear to control gene expression through multiple mechanisms. It has become clear that one of these mechanisms is the promotion of methylation—siRNAs have previously been found associated with methylated sites, and the authors recently showed that siRNAs could direct DNA methyltransferase to tandem repeats.



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Tandem repeats recruit siRNA production and DNA methylation in two steps. (Image: Simon Chan)

In *Arabidopsis*, the lab rat of the plant world, there are two tandem repeats near the beginning of a well-studied gene called *FWA* which are targeted for methylation. *FWA* is a good model for studying methylation, because when unmethylated *FWA* is inserted into *Arabidopsis*, 100% of the introduced genes become methylated, far more than other genes. When *FWA* is methylated, the plant flowers early. Mutants that leave the gene unmethylated flower late.

The authors first showed that the *FWA* tandem repeats are integral to triggering new methylation. An unmethylated *FWA* gene introduced into *Arabidopsis* plants that themselves had unmethylated *FWA* (and therefore flowered late) caused a portion of the transformed plants to flower early. This indicated that somehow the introduced gene triggered methylation of the endogenous *FWA* gene, as well as of itself (the unmethylated form is dominant, and so would stimulate late flowering unless it too had become methylated). When the tandem repeats were deleted from the introduced gene, the effect was lost. And when the tandem repeats alone, minus the rest of the gene, were introduced, the endogenous gene again became methylated and silenced, and flowering occurred early. Together, these results show the tandem repeats are both necessary and sufficient to stimulate methylation.

To test whether it was the mere sequence of the repeats, or rather their double nature, that promoted methylation, the authors introduced a gene containing only one member of each tandem-repeat pair into plants with the nonmethylated form. No methylation took place, and the plants again flowered late. Thus, it appears that the sequences must be present as tandem repeats to direct methylation to *FWA*.

What was the mechanism by which the introduced gene triggered methylation? The authors have elsewhere shown that absence of any of the multiple factors responsible for synthesizing siRNA produces the same late-flowering phenotype, suggesting that siRNA is intimately connected to the methylation process. To find out, they examined the methylating ability of multiple plant lines, each a mutant for one or more genes in the siRNA methylation pathway, and also

carefully measured the production of siRNAs complementary to the *FWA* tandem repeats (no mean feat, since their abundance is less than a tenth of one percent that of some other RNA species). They found that plants unable to methylate DNA could still produce siRNAs, while those that could not produce siRNAs could not carry out methylation. In addition, they made the surprising finding that siRNAs are produced from the unmethylated *FWA* gene. These findings show that siRNA-directed methylation is a two-step process, in which recruitment of siRNA production precedes recruitment of DNA methyltransferase.

Based on their results, the authors propose that tandem repeats act as attractors for the siRNA-making complex. Production of siRNA from these sequences then attracts the methylating machinery, leading to silencing of the gene containing the repeat. In this way, the gene regulatory apparatus functions somewhat like a genomic immune system, identifying potential threats and neutralizing them. As further evidence for this model, the authors showed that throughout the *Arabidopsis* genome, methylation of tandem repeats occurred at a much higher frequency when those repeats were associated with siRNAs than when they were not. Further exploration of this gene-silencing system may help explain how our genomes have been immunized against the ravages of parasitic DNA over life's history, a process that continues into the present.

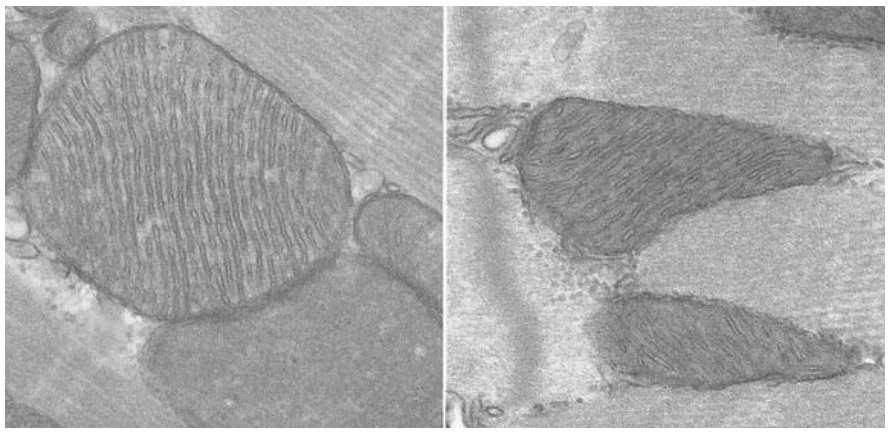
Chan SWL, Zhang X, Bernatavichute YV, Jacobsen SE (2006) Two-step recruitment of RNA-directed DNA methylation to tandem repeats. DOI: 10.1371/journal.pbio.0040363

PGC-1β: A Regulator of Mitochondrial Function with Subtle Roles in Energy Metabolism

Françoise Chanut | DOI: 10.1371/journal.pbio.0040402

The evolutionary success of mammals is owed in part to their ability to adjust their metabolism to the demands of their environment. For instance, mice faced with dropping external temperatures crank up the output of a specialized type of fat—called brown adipose tissue (BAT) —that generates heat. Faced with a lack of food, they turn on the production of glucose, a highly energetic sugar, by the liver. Both of these adaptive responses depend on a nuclear protein called PGC-1α. PGC-1α simultaneously increases the expression of genes acting at multiple steps along the heat- or glucose-production pathways, thereby quickly boosting the pathways' efficacy. A related protein, PGC-1β, shares many of PGC-1α's functional characteristics: for instance, both proteins can, when overexpressed in mice, increase the activity of mitochondria, the intracellular organelles that turn sugars and fats into heat or the cellular fuel ATP. To determine what role PGC-1β normally plays in energy metabolism, Christopher Lelliott, Gema Medina-Gomez, Antonio Vidal-Puig, and their colleagues created mice lacking the PGC-1β gene. Their recent study of the resulting phenotype shows that PGC-1β has different functions than PGC-1α and plays a subtler role than PGC-1α in energy metabolism.

As expected from PGC-1β's ability to stimulate mitochondrial function, the loss of PGC-1β decreased the transcription of genes encoding many of the mitochondrial proteins that generate ATP and heat. Yet the mutant mice appeared essentially healthy under normal conditions. Still, a slight impairment of mitochondrial function might lead to some metabolic imbalance, for instance obesity, since mitochondria burn energy that would otherwise be stored as fat. But the mutant mice were in fact smaller and leaner than their wild-type counterparts. This phenotype did not reflect differences in appetite or digestion, since the mutants ate and eliminated similar amounts of food as wild types. Instead, the mutants had higher metabolic rates than wild types at ambient temperature: they burnt more calories than wild types did to sustain basal physiological functions such as respiration, heartbeat, and body temperature, which would suggest that their mitochondrial functions were increased, rather than decreased.



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The typical mitochondrial density within hearts of normal mice (left panel) becomes considerably reduced in mice lacking the gene for PGC-1β (right panel).

The explanation for these counterintuitive findings is that loss of PGC-1β led to increased expression of PGC-1α in fat tissues. The researchers propose that the increase in PGC-1α, which can stimulate heat production in BAT, compensates for the loss of PGC-1β at ambient temperature. To remove the compensatory effect of PGC-1α, the researchers measured the metabolic rate of mutants housed at 30 °C, a temperature close to body temperature, at which PGC-1α expression is largely abolished. At this temperature, mice lacking PGC-1β had a reduced metabolic rate, suggesting that PGC-1β can contribute to maintaining normal metabolic rates.

The mutant mice were able to withstand and adapt to cold, presumably because cold temperatures could still trigger PGC-1α expression. However, when mutant mice that were acclimatized to 4 °C were further stimulated by norepinephrine, a neurotransmitter that normally induces BAT to respond to cold, their BAT heat output was blunted compared to that of wild-type BAT. Therefore, PGC-1β must normally play a role in thermal regulation, though its role is obscured by PGC-1α's induction at low temperatures.

In other tissues such as liver, muscle, and heart, loss of PGC-1β did not trigger PGC-1α expression, which made the role of PGC-1β easier to discern. In the liver, PGC-1β proved necessary for the proper disposal of cholesterol and other lipid forms when mice were fed a diet rich in saturated fat. In muscles and heart, PGC-1β was found necessary to maintain a normal number of mitochondria. But even with a reduced mitochondrial count, the hearts of mice lacking PGC-1β functioned normally. Their only anomaly was a failure to increase their pulse rate properly when challenged with the heart stimulant dobutamine. By comparison, mice lacking PGC-1α develop severe heart failure.

These observations show that although PGC-1β and PGC-1α share sequence and functional similarities and are expressed in the same tissues, they cannot completely substitute for one another. The researchers propose that PGC-1β maintains basal metabolic functions, whereas PGC-1α allows the body to step up it response to high energetic demands.

Lelliott C, Medina-Gomez G, Petrovic N, Kis A, Feldmann HM, et al. (2006) Ablation of PGC-1β results in defective mitochondrial activity, thermogenesis, hepatic function, and cardiac performance. DOI: 10.1371/journal.pbio.0040369

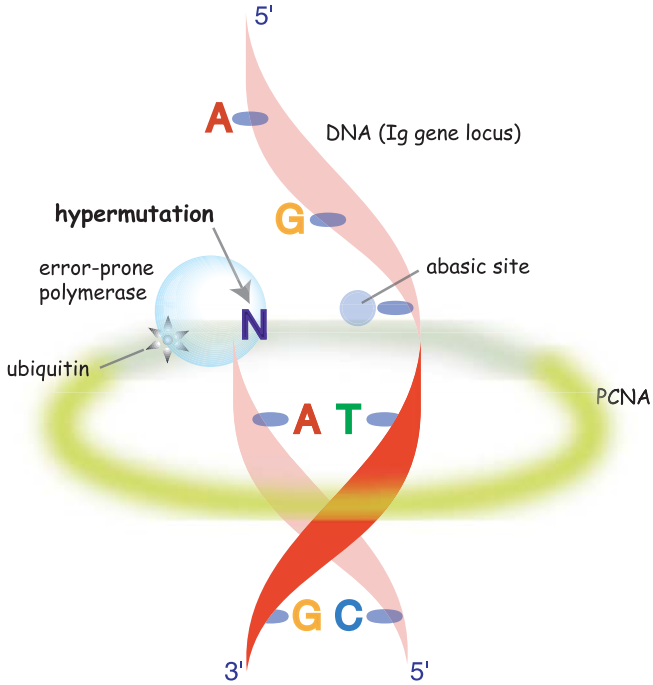
Borrowing a DNA Repair Enzyme to Fine-Tune Antibody Specificity

Liza Gross | DOI: 10.1371/journal.pbio.0040403

When immunologists discovered in the 1970s that human immune cell receptors recognize tens of millions of antigens—telltale signs of a foreign invader—they were mystified. How could a genome with just 100,000 genes (now estimated at fewer than 25,000) generate this staggering diversity? The answer came with the discovery of a gene rearrangement process that draws on millions of possible permutations to create a unique surface receptor on every T and B lymphocyte—the immune system's main weapons. But gene diversification of B lymphocytes doesn't stop there. The immunoglobulin genes, which encode the Y-shaped antibodies that flag infectious microbes for destruction, undergo additional point (single–base pair) mutations that enhance pathogen recognition.

In a new study, Hiroshi Arakawa, Stefan Jentsch, and Jean-Marie Buerstedde find a surprising link between one of these mutation-inducing mechanisms, called immunoglobulin hypermutation, and a DNA repair pathway. When the transcription machinery encounters a DNA lesion, the RAD6 pathway (named after the *Rad6* DNA-repair gene) steps in to either recruit specialized translesion polymerases to bypass the damage, or to copy the correct base sequence from the intact sister strand. While adept at dealing with damaged DNA, the translesion polymerases are "error-prone" compared to standard replicative DNA polymerases. This tendency can be beneficial within the immunoglobulin genes, however, because the introduced mutations can improve antigen recognition of the encoded antibodies. But it was unclear how translesion polymerases are recruited for immunoglobulin hypermutation.

Studies in yeast show that *Rad6* and *Rad18* tag proliferating cell nuclear antigen (PCNA) with a small molecule called ubiquitin. The tag is added to a conserved lysine amino acid residue, called K164—a ubiquitination target in eukaryotes from yeast to humans. Yeast PCNA can undergo modifications at K164 by one ubiquitin tag (mono-ubiquitination), multiple tags (poly-ubiquitination), or by small, ubiquitin-related modifiers (SUMOs) in response to DNA damage. An amino acid substitution from lysine (K) to arginine (R) at position 164 (K164R) in yeast prevents the ubiquitination and SUMOylation but does not compromise the functions of the unmodified PCNA.



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The authors show that vertebrates exploit the PCNA-ubiquitin pathway—a DNA repair pathway—for immunoglobulin hypermutation, most likely through the recruitment of error-prone DNA polymerases.

The role of PCNA ubiquitination has been well characterized in yeast but not in higher eukaryotes. It had been reported that human PCNA undergoes only mono-ubiquitination at K164, which increases its affinity for two translesion polymerases, Polη and REV1. Working with a chicken B cell line whose genetic tractability has made it a favored model for studying DNA repair and immunoglobulin hypermutation, the authors generated a series of clones carrying the $PCNA^{K164R}$ mutation

either alone or in combination with mutations that inactivated the *RAD18* or *REV1* genes.

The authors analyzed extracts from the progenitor line and mutant clones to look for PCNA modifications. Mono-ubiquitinated and "SUMOylated" PCNA was evident in nonmutant cells, but clones carrying the $PCNA^{K164R}$ mutation showed neither modification. PCNA mono-ubiquitination was markedly reduced, but not eliminated, in cells lacking functional *RAD18* genes. The $PCNA^{K164R}$ mutation also made cells vulnerable to DNA-damaging agents—likely because PCNA failed to recruit the translesion polymerases—suggesting that vertebrates also require PCNA ubiquitination at the K164 site to survive DNA damage.

All the clones under study expressed immunoglobulin on their surface, allowing the authors to easily track the appearance of harmful mutations in the immunoglobulin variable regions (the targets of hypermutation). Whereas the progenitor line (serving as a control) showed a high rate of immunoglobulin loss (about 35% after 2 weeks culture), the rate was 7-fold reduced in the $PCNA^{K164R}$ mutant clone. Loss of *RAD18* or *REV1*, however, reduced the rates of mutations by about 2-fold and 3- to 4-fold, respectively.

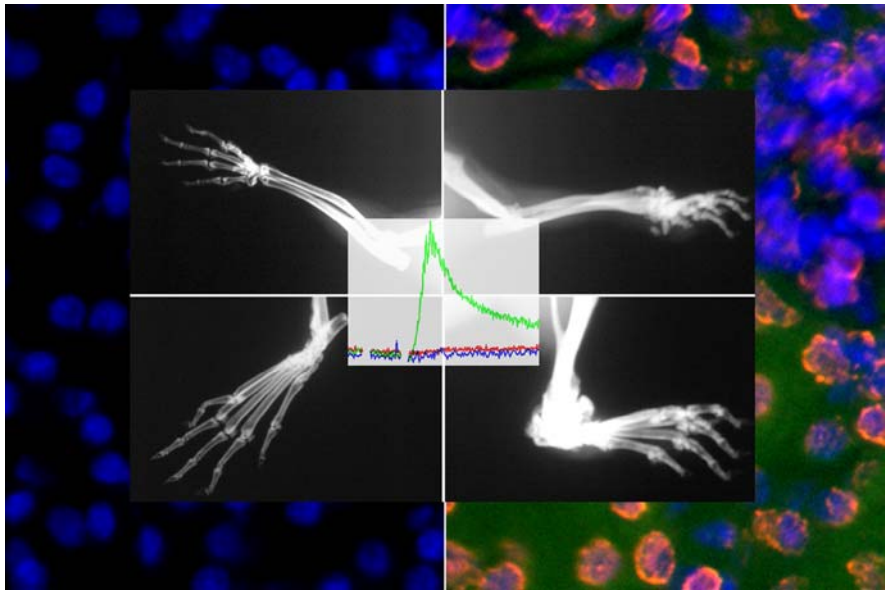
These results demonstrate that the $PCNA^{K164R}$ single amino acid substitution not only renders cells sensitive to DNA damage but also dramatically impairs their capacity for immunoglobulin hypermutation. Both effects most likely result from the absence of ubiquitination, since the *RAD18* mutant clone displays a similar but less severe DNA repair and hypermutation defect. Now that it's clear that the immune system has tailored the ubiquitination-PCNA pathway to its own needs, researchers can begin to work out the molecular details of this appropriation—and perhaps explain how vertebrates managed to maximize the benefits of one pathway by using it in two systems critical for the survival of the organism.

Arakawa H, Moldovan GL, Saribasak H, Saribasak NN, Jentsch S, et al. (2006) A role for PCNA ubiquitination in immunoglobulin hypermutation. DOI: 10.1371/journal.pbio.0040366

Immune Cells Strike a Balance to Avoid Autoimmune Disease

Mason Inman | DOI: 10.1371/journal.pbio.0040393

Immune cells walk a fine line, biochemically speaking. They must be sensitive enough to recognize foreign invaders like bacteria and viruses, but not so reactive that they attack an animal's own tissues, paving the way for autoimmune disorders. Animals use a variety of mechanisms to strike this balance. One way is by regulating the activity of T cells—immune cells that can trigger an immune response to billions of different molecules—through a range of processes. In one process known as deletion, autoreactive T cells (which attack the body) are killed off. Another process involves making a subset of T cells develop into a specialized type, called suppressor T cells (or T regulatory cells). The suppressor T cells can then regulate the behavior of other immune cells, damping down autoimmune reactions.



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Self-reactivity of T cells can be down-regulated by cell-intrinsic adaptation (as indicated by reduced calcium fluxes of adapted T cells shown in the center inset). Left and right panels show samples from mice with and without autoreactive T cells, respectively. (Image: Nevil Singh et al.)

In addition to these two well-studied mechanisms for avoiding autoimmunity, evidence is mounting that animals have another tool at their disposal, a process called adaptation. This process seems to come into play especially when the immune system faces a persistent infection or continually reacts to the body's own tissues. In these cases, avoiding a fatal autoimmune response requires keeping the immune system in check. In adaptation, immune cells become habituated to foreign (or apparently foreign) proteins and tone down their activity. But how adaptation works together with deletion and regulation by suppressor T cells to avoid autoimmunity has been difficult to tease out.

Nevil Singh, Chuan Chen, and Ronald Schwartz studied adaptation in isolation from the other mechanisms for avoiding autoimmunity and found support for the view that adaptation is a mechanism independent of either deletion or regulation by suppressor T cells. The researchers injected T cells into a strain of mice that were engineered to lack their own T cells. The injected T cells hadn't yet encountered any foreign or activating proteins (and so are called "naive" T cells). Singh and colleagues could watch the injected T cells adjust to their new environment, and because the host animals had no T cells of their own, the naive T cells could adjust without interference from native T cells that had matured in the mice's bodies.

The researchers found that the injected T cells multiplied, as they do in mounting an immune response to something foreign, but then over about a week, adapted to the new environment. The injected T cells toned down their activity, but persisted in the animals' bodies for more than two months (without invoking deletion). They did not develop into suppressor T cells, either.

Despite the adaptation of the injected T cells, they caused the mice to develop arthritis, an autoimmune disease, after a few months. This was a surprise, since the T cells appeared to have adjusted to being in the mice. To pin down how the T cells caused this autoimmune response, the researchers injected naive T cells into another mouse strain, which lacks B cells—immune cells that create antibodies against foreign proteins. The B cells typically do this only after being triggered by T cells that have recognized foreign cells. The researchers let the T cells adapt to being in the mice, and then introduced B cells from outside. Surprisingly, although the adapted T cells seemed to tolerate the body's tissues, they still retained the ability to activate B cells, which then produced a variety of antibodies against the body's own tissues, eventually leading to arthritis.

In a final set of experiments, Singh and colleagues put the naive T cells into adult mice of a strain born supplied with their own T cells. In this case, contrary to the earlier experiments, the naive T cells did not give the mice arthritis or produce other signs of an autoimmune response. The T cells still undergo adaptation but are subsequently deleted slowly. Interestingly, in the presence of a full set of T cells, the autoreactive T cells also developed fewer effector functions.

Together, these results suggest that to keep T cells from developing in a way that promotes autoimmunity, two kinds of regulation are necessary. First, there's the adaptation of the T cells to other cells that they see for a long time. And second, there's the regulation that comes with competing among a variety of T cells that have gone through normal development in the animal. The authors make the case that these different types of regulation have to be understood separately, making a step toward better understanding how autoimmune disorders arise.

Singh NJ, Chen C, Schwartz RH (2006) The impact of T cell intrinsic antigen adaptation on peripheral immune tolerance. DOI: 10.1371/journal.pbio.0040340

New Factors Controlling Parent-Specific Genetic Control

Jason Underwood | DOI: 10.1371/journal.pbio.0040398

In humans, two linear meters of DNA must miraculously compact down and fit into each cell's nucleus. Special proteins known as histones act as the spools around which DNA is coiled and contorted. This system keeps the genome restricted to a reasonable space and also allows for dynamic changes in gene regulation. Different regions of the DNA can become decondensed and activated in accordance with developmental timing, cell type, or in response to the environment. Some regions of the genome remain silent for the life of the organism, while others must respond at the flip of a switch, turning particular genes on or off in response to cellular cues.

Humans and other animals have diploid genomes, meaning that they have two versions of each gene, one from each parent. These two copies, or alleles, can be regulated in concert or independent from one another. Genetic imprinting is a special case where gene expression is restricted to just one of the parental alleles. One interesting and well-studied example of imprinting occurs in a region of the genome where the neighboring genes Igf2 and H19 reside. The gene for Igf2, an insulin-like growth factor, is only expressed from the paternal allele, while the noncoding RNA gene, H19, is only expressed from the maternal allele. A small DNA region in between the two genes, appropriately called the imprinting control region (ICR), assigns the neighboring gene's activity. The paternal allele ICR has small chemical modifications on the DNA known as methylation, and this is key to proper Igf2/H19 regulation. The mechanism by which only the paternal allele gets these modifications has long remained a mystery, but now a recent study indicates a link between a testis-specific protein and the paternal methylation of the ICR. The study by Petar Jelinic, Jean-Christophe Stehle, and Phillip Shaw demonstrates that in mice, this testis-specific factor, CTCFL, binds to the ICR and recruits other factors and enzymes that direct the methylation of this region.

The factor of interest, CTCFL, was discovered several years ago and became an interesting candidate for regulation of the Igf2/H19 region not only because of its testis expression pattern, but also because its amino acid sequence resembles another known DNA-binding protein, CTCF. This protein was known to bind to specific DNA sequences present in the ICR. As expected, the testis protein, CTCFL, could also bind to the same sequences. Then, the CTCFL protein was used as bait in a genetic fishing expedition to catch proteins that might physically interact with CTCFL. Interestingly, the two "fish" that were caught were both factors that are known to play key roles in gene regulation. One was a testis-specific component of the DNA-spooling complexes, a histone H2A protein variant. The other protein was an enzyme that can add methyl groups to other proteins. This enzyme, protein arginine methyltransferase 7 (PRMT7), was previously shown to add methyl groups to histone proteins, and these methyl modifications can have profound effects on the activity of the bound DNA region.

These new candidates for Igf2/H19 regulation were tested in a number of assays. After confirming that CTCFL proteins can physically bind the PRMT7 enzyme and histone proteins, the authors verified that they are expressed in the testis during the proper developmental stages to influence imprinting. Next, the authors used clues from previous studies on PRMT7, which indicated several candidate methyl targets of particular interest to gene regulation gurus, the histone proteins. Since CTCFL can bind to the enzyme and

to histones, the authors postulated that perhaps CTCFL acts as a meeting place for the enzyme and the histones. Their observations were in agreement with this hypothesis, as they found that CTCFL significantly stimulated the efficiency with which PRMT7 could methylate the histone family members H2A and H4. But, could the activity of these factors and the methylation of the histones affect the methylation of the DNA itself, since it is this type of modification that tags the paternal allele? To address this question, the candidate genes as well as genes for the methyl transferring enzymes that work on DNA were injected in various combinations into frog eggs. A DNA sequence that resembles the mouse ICR region was also injected into the eggs, and a special assay was used to monitor the methylation of this foreign DNA. When all three key players—CTCFL, PRMT7, and the enzyme that methylates DNA, Dnmt3—were injected, significant DNA methylation was observed on the ICR sequence. Leaving out any of these components resulted in lower overall methylation. Taken together, it appears that these new components play important roles in paternal imprinting.

The authors propose a model to account for the methylation events on both histone proteins and on the ICR DNA. This study provides two new factors to tweak this fascinating genetic event at the Igf2/H19 region, but it is clear that this model will become even more complex in time. This region is arguably the best studied model for imprinting, and yet there's so much left to be learned about these mysterious control mechanism guarding and prying at the genome.

Jelinic P, Stehle JC, Shaw P (2006) The testis-specific factor CTCFL cooperates with the protein methyltransferase PRMT7 in *H19* imprinting control region methylation. DOI: 10.1371/journal.pbio.0040355

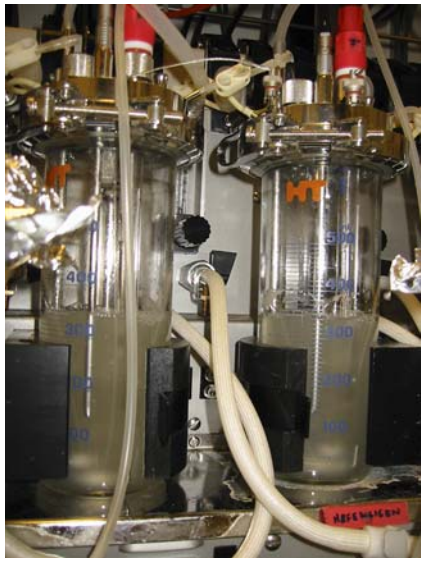
An Unexpected Connection: Potassium Limitation and Ammonium Toxicity in Yeast

Mary Hoff | DOI: 10.1371/journal.pbio.0040389

Give the yeast *Saccharomyces cerevisiae* the right growing conditions and it multiplies like crazy—as any bread maker or beer brewer can testify. But deprive it of sufficient potassium, and it's lucky to survive. Why? Since *S. cerevisiae* is a model organism for eukaryotes, the answer to that question could provide valuable insights into cellular processes of many organisms, including humans.

To learn how potassium limits growth in yeast, David C. Hess, David Botstein, and colleagues enlisted the assistance of DNA microarray analysis, a biochemical tool that allows scientists to identify which genes are active in a cell at any given time. What they found gave them a start: in *S. cerevisiae* cells grown in a potassium-limited medium, genes involved in a seemingly unrelated process—nitrogen metabolism—showed dramatically altered activity compared with unstressed cells, with genes repressed by products of nitrogen-compound breakdown becoming less active, and those whose products facilitate amino acid transport showing increased activity.

At first, that made about as much sense as discovering that every time you open your refrigerator the stock market drops. What could possibly be the connection? The altered gene activity pattern suggested an attempt to deal with a toxic influx of nitrogen within the cell, but nitrogen toxicity has been thought to be limited to multicellular organisms, with one-celled types easily able to keep the nutrient in balance by excreting excess through cell membrane channels. Could limited potassium upset that ability?



DOI: 10.1371/journal.pbio.0040389.g001

By applying systems-level biology to yeast cells growing in steady-state potassium limited chemostats (pictured above), the authors uncovered ammonium toxicity in yeast. (Image: Maitreya Dunham)

In search of an answer, the scientists looked at cells exposed to different ammonium and potassium levels. They found that in low-potassium but not high-potassium environments, cell numbers went down dramatically as ammonium concentration increased, suggesting that ammonium is indeed toxic to yeast when potassium is limited. A second test, in which they increased concentration of the nitrogen-rich amino acid asparagine rather than ammonium, confirmed that what they were seeing was not a general nitrogen effect, but one specific to ammonium. Further tests of other strains of *S. cerevisiae* confirmed that they were not dealing with a situation unique to a single quirky cell type.

If what they were seeing was indeed an adverse reaction to ammonium, the researchers predicted they should also see some sort of metabolic fingerprint of the yeast's efforts to detoxify its environment. And they did. In collaboration with the Rabinowitz lab at Princeton, they used liquid chromatography tandem mass spectrometry to test the biochemical contents of medium in which ammonium-stressed yeast cells were grown. There, the researchers found high levels of amino acids—apparently the yeast equivalent of the urea we mammals excrete in urine to remove toxic nitrogen from our system.

Having confirmed the presence of ammonium toxicity, the researchers next turned their attention to the issue of the mysterious connection with potassium concentration. Because potassium and nitrogen have similar chemical properties, they hypothesized that ammonium ions leak into cells through potassium channels when those channels are not otherwise

occupied ushering potassium across the cell membrane. To test this, they engineered strains of *S. cerevisiae* in which ammonium influx into the cells could be increased without stimulating innate ammonium concentration regulatory mechanisms. Even in high-potassium environments, cells engineered to let in lots of ammonium showed greater mortality than those engineered to let in little, supporting the hypothesis that excess influx of ammonium is the root of the problem. Furthermore, the researchers found that in engineered cells in which ammonium transport across the cell membrane was high, growth was indeed limited even though potassium was not, and the cells excreted high levels of amino acid, mimicking the potassium-limited state.

The researchers concluded that *S. cerevisiae* does indeed experience ammonium toxicity under potassium-deprived conditions and that it uses a primitive detoxification system involving the production and excretion of amino acids in an attempt to deal with it. On a broader level, they demonstrated that systems biology techniques such as microarray analysis and mass spectrometry are valuable resources for discovering and exploring biochemical relationships and pathways that might otherwise remain masked in the normal workings of healthy cells. They hope in further studies to use these and other emerging tools to learn whether similar ammonium toxicity is also found in bacteria and to elucidate the mechanism behind *S. cerevisiae*'s amino acid–based detoxification system. For more on ammonium toxicity, see the related Primer (DOI: 10.1371/journal.pbio.0040388).

Hess DC, Lu W, Rabinowitz JD, Botstein D (2006) Ammonium toxicity and potassium limitation in yeast. DOI: 10.1371/journal.pbio.0040351

Metagenomics Offers a Big-Picture View of the Diversity and Distribution of Marine Viruses

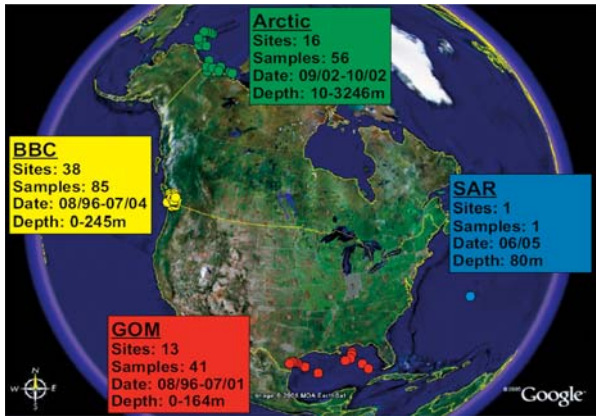
Mary Hoff | DOI: 10.1371/journal.pbio.0040406

When we think of ocean life, we tend to think of sharks and squid and sea turtles and such. Underpinning these large life forms is a massive but much less conspicuous world of microscopic bacteria and archaea. And existing at an even lower rung, bridging the gap between life and nonlife, are bacteriophage (phage) viruses—minute, self-replicating bundles of biochemicals that alter microorganisms' genetic material and moderate their communities through predation and parasitism. Although unfathomably tiny, marine bacteriophages are also astoundingly abundant—there are about as many of them in a bucket full of seawater as there are humans on the planet. As a result, they can have a cumulatively huge impact as they individually alter the flow of energy, biomass, and genes through the biosphere.

To get a better picture of the bacteriophage bounty found in marine environments, Florent Angly, Forest Rohwer, and colleagues used metagenomics, an approach that applies genomic techniques to large samples, rather than to individual organisms. By providing a snapshot of the DNA of uncultured viruses in the oceans, metagenomics offers valuable insights into viral diversity, geographical distribution, taxonomic composition, and ecosystem functioning.

The subjects of study were 184 water samples collected from 68 sites over 10 years' time from four ocean regions: the Sargasso Sea, the Gulf of Mexico, British Columbia coastal waters, and the Arctic Ocean. Each sample was analyzed using a new DNA sequencing technology called pyrosequencing, which makes it possible to obtain a large number of DNA sequences (albeit small ones) at a lower cost than conventional sequencing approaches, to determine the nature of the viral DNA present. The resulting viral metagenomes, or viromes, were compared with a large public database of genomes that have been sequenced, with an "environmental database" consisting of genomes found previously in diverse natural settings, and with an existing database of viral genetic material.

The innovative approach yielded a picture of tremendous diversity in the viral composition of the oceans, with more than 91% of the DNA sequences found differing from the known databases. The genomes found included those of cyanophages, several unusual viruses, and a single-stranded DNA phage—the first of its kind found in abundance in the marine environment—suggesting a unique "marine-ness" in the viral composition of ocean water.



DOI: 10.1371/journal.pbio.0040406.g001

The authors used metagenomics to analyze the "viromes" of oceanic viruses and shed light on their diversity, distribution, and ecosystem impact in four ocean regions around the world.

Due to their size and lack of locomotion, viruses are believed to be easily dispersed by marine currents or even sea breezes. The researchers used three statistical approaches to analyze the distribution of marine phages among sampling sites. They found that the distribution among the sample sites was not random and that it tended to differ not only among ocean regions, but also compared with the distribution of land-based viruses. They also showed a correlation between geographic distance and genetic distance between viral species, supporting the contention that the marine virome varies from region to region, even though many species are found at more than one sampling site. Finally, to assess how much the viral makeup

of various environments overlaps, the researchers mixed the DNA sequences from the four regions and observed the extent to which fragments with different origins meshed with each other—an indicator of the similarity of the viromes. A simulation of this data suggested that the differences among the regions was mostly explained by variations in relative abundance of the predominant viral species, rather than by the range of viruses present at each site. This supports the saying that "Everything is everywhere, but, the environment selects."

So, how diverse is the viral makeup of the marine environment? Samples taken off the British Columbia coast were the most genetically diverse—not surprising, since an upwelling in the area offers a nutrient-rich environment for supporting a wide range of life forms upon which viruses depend. The other three samples showed increasing diversity with decreasing latitude, a trend that parallels previous findings from terrestrial ecosystems. Extrapolating from their observations, the researchers predicted that the world's oceans hold a few hundred thousand broadly distributed viral species, with some species-rich regions likely harboring the majority of these species.

In addition to analyzing their results, the researchers commented that they obtained and combined multiple samples in space and time from all but the Sargasso Sea site, because they thought this would provide the best approximation of the actual meta-viral profiles. The data analysis of the single Sargasso Sea sample, however, led them to conclude that individual samples at the other sites might have led to equally representative results. Such a sampling approach, they noted, would yield additional benefits in the form of opportunities to explore spatio-temporal gradations not discernable using the integrative sampling approach. Other changes they proposed to further expand the usefulness of viral metagenomic analysis include expanding sampling capability to include large DNA viruses and finding a way to include RNA viruses. The researchers are looking forward to future studies that will further characterize the viral makeup of the oceans and other unsequenced environments, including ones that explore the nature and the implications for ecosystems of marine viruses' relationship with their microbial hosts.

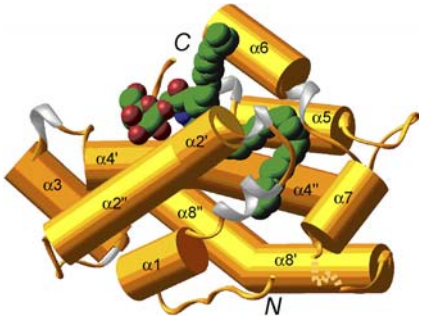
Angly F, Felts B, Breitbart M, Salamon P, Edwards R, et al. (2006) The marine viromes of four oceanic regions. DOI: 10.1371/journal.pbio.0040368

Too Long, Too Short, or Just Right: Glycosphingolipid–Protein Binding Varies with Acyl Chain Length

Richard Robinson | DOI: 10.1371/journal.pbio.0040397

Glycosphingolipids (GSLs) reside in the membranes of all mammalian cells, where they play roles in both structure and signaling. They traffic between the plasma membrane—where most are found—and vesicle membranes within the cell. One of the carriers of GSLs is glycolipid transfer proteins. The interactions between these two molecules have only recently begun to be elucidated. In a new study, Lucy Malinina, Margarita Malakhova, Rhoderick Brown, Dinshaw Patel, and colleagues reveal a highly unusual binding characteristic of the protein: the sphingosine chain of the GSL either buries itself inside the protein or is left outside of it, depending on the length of the acyl chain.

Every GSL has three parts: a sugar head and two long hydrocarbon chains (an 18-carbon, nitrogen-containing sphingosine chain, and an "acyl" chain whose length can vary from 16 to 26 carbons). Using x-ray crystallography, the authors recently elucidated the structure of human glycolipid transfer protein, both with and without an attached GSL, and showed that it has a novel protein fold adapted to interacting with membranes and binding with lipids. In that study, which used a GSL containing a lactose sugar and an 18-carbon monounsaturated acyl chain, they found that the sugar binds to the exterior, while the sphingosine and acyl chains lay parallel inside a hydrophobic tunnel made from an interior fold of the protein. To explore how the protein accommodated other GSLs, they varied acyl length and sugar groups and determined the structure of these protein–GSL complexes.



DOI: 10.1371/journal.pbio.0040397.g001

The sphingosine chain of GSL is blocked from entering the tight confines of the GLTP hydrophobic tunnel because the long acyl chain, which enters first, is forced into a serpentine-like conformation within the tunnel.

To their surprise, they found that when the acyl chain was either longer (24 carbons) or shorter (8 or 12 carbons) than the one in their initial experiment, the sphingosine chain was not included in the tunnel, but instead jutted out away from the surface of the protein. While the effect on sphingosine is the same, the cause appears to be slightly different in the two cases. When the shorter acyl chain sits in the tunnel, it is joined by an extraneous free hydrocarbon, which denies sphingosine an entrance. The exact origin and role of this hydrocarbon is unknown, but it also occupies the tunnel in the unbound protein. In contrast, there is no extraneous hydrocarbon when the longer acyl chain is in the tunnel, but the chain curls around within, apparently blocking out sphingosine with its bulk. When the authors reverted to the 18-carbon acyl chain but introduced an additional chain-kinking double bond, once again sphingosine was excluded, suggesting that its ability to fit depends on both the length and shape of the acyl group. The tunnel itself expands and contracts with the changes in size of the chains within.

Unlike the highly variable interactions of tunnel and hydrocarbon chains, the binding of sugar to the protein appears to rely mainly on a small set of invariant attractions, whether from the double sugar, lactose, or from the single sugars, galactose or glucose. In addition, in each case there are conserved hydrogen bond contacts involving an amine and carbonyl (amide linkage) in the GSL ceramide and specific amino acids of the protein, helping to position the GSL hydrocarbons for entry into the tunnel. The binding of the amide group also triggers a conformational shift in one loop of the protein at the head of the tunnel.

From these observations, the authors propose a stepwise binding sequence for GSLs, in which the sugar binds first, acting as the primary determinant of GSL-protein specificity. The amide group binds next, orienting the GSL tails with the tunnel and shifting the loop to help open the tunnel for the hydrocarbons. The acyl hydrocarbon binds next. The sphingosine follows the acyl group into the tunnel in some cases, while in others, it remains outside.

These results should accelerate understanding of GSL trafficking within the cell and provide insight into how these molecules play their parts in cell growth and development.

Malinina L, Malakhova ML, Kanack AT, Lu M, Abagyan R, et al. (2006) The liganding of glycolipid transfer protein is controlled by glycolipid acyl structure. DOI: 10.1371/journal.pbio.0040362

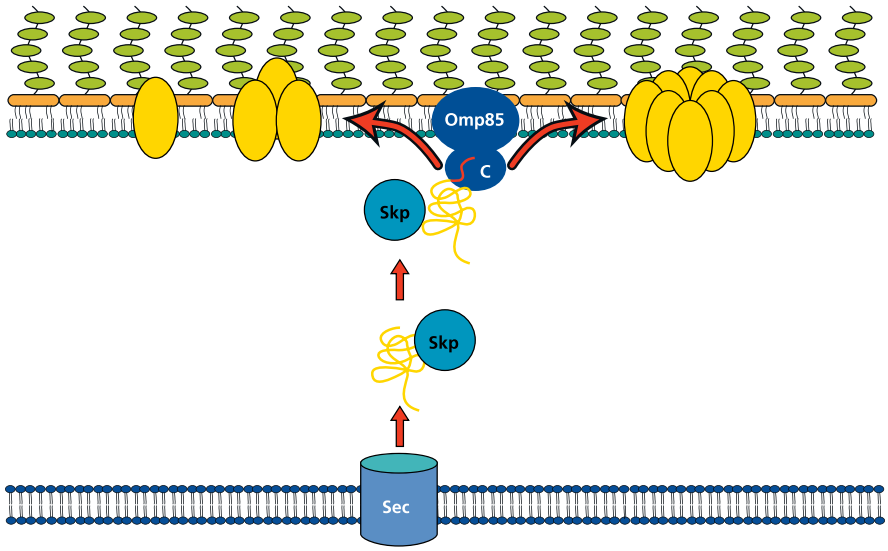
Picking the Right Parts at the Beta Barrel Factory

Mary Hoff | DOI: 10.1371/journal.pbio.0040399

Tube-shaped protein constructs known as beta barrels are used by Gram-negative bacteria as passageways for molecules that need to travel from one side of the outer cell membrane to the other. Getting beta barrels embedded into the membrane is a daunting task because of the nonpolar nature of the membrane's insides; the way cells manage that is with the assistance of a tubular factory already embedded in the membrane that takes in and assembles the outer membrane proteins (OMPs) that comprise the beta barrel. A protein called Omp85 is a central component of this factory in a surprising number of bacterial species as well as in mitochondria and chloroplasts (eukaryotic organelles that arose from bacteria).

To learn about the structure and function of this evolutionarily conserved part of the beta barrel factory, Viviane Robert, Jan Tommassen, and colleagues reconstituted *Escherichia coli* Omp85 in vitro, then looked at how it interacted with the OMPs that assemble to form beta barrels.

When placed in an artificial membrane, the reconstituted Omp85 formed a voltage-activated channel—a property that could be used to study its interaction with substrate OMPs. Indeed, when the researchers exposed their construct to denatured OMPs, they found an interaction reflected by increased Omp85 channel activity, presumably to begin the beta barrel assembly process. But they also found that it didn't interact with other proteins they tested. What, they wondered, is the secret password OMPs use to access the Omp85 factory?



DOI: 10.1371/journal.pbio.0040399.g001

Planar lipid bilayer experiments revealed that the evolutionarily conserved assembly factor Omp85 recognizes the signature motif present at the C termini of bacterial OMPs.

Previous studies had shown that a "signature sequence" of amino acid residues on the C terminus of OMPs—consisting of a phenylalanine or tryptophan at the very end, and hydrophobic residues in the 3, 5, 7, and 9 positions from the C terminus—is important to cell survival in bacteria that use Omp85 to make beta barrels. That led the researchers to hypothesize that the signature sequence is key to gaining entry into Omp85. To test their hypothesis, they looked at a mutant OMP that lacked the proper C-terminal signature sequence. The mutant OMP not only was not admitted by the Omp85, but at high concentrations, it even blocked the Omp85 channel completely. In further support for their hypothesis, the researchers manufactured an imposter—a synthetic peptide with the appropriate C-terminal signature sequence—and presented it to the Omp85. As they predicted, it stimulated the channel to open, providing additional evidence that Omp85 indeed recognizes OMPs by their C-terminal signature sequence.

Since Omp85 is found in a variety of bacteria, the researchers decided to test whether the C-terminal signature sequence key from one species would unlock the Omp85 gates in another. They found that neither PorA (an OMP from *Neisseria meningitidis*) nor its C-terminal peptide pushed the right buttons for *E. coli* Omp85, even though their C-terminal signature sequence

is similar to that of *E. coli* OMPs. This fits with previous observations that the presence of *N. meningitidis* OMPs is fatal to *E. coli*, but it also raises the question as to what the discriminating characteristic might be. To find out, the researchers compared C-terminal sequences of *N. meningitidis* and *E. coli* OMPs. They discovered that *N. meningitidis* OMPs tend to have arginine or lysine residues at position 2 from the C-terminus, while *E. coli* OMPs do not. Further testing of OMPs with various amino acid residues in the penultimate position provided further support for their speculation that that particular residue is responsible for the species specificity they observed.

The researchers concluded that the use of an Omp85 factory to get OMPs into the outer membrane is conserved across species, but some differences in recognition of appropriate OMPs have evolved since the organisms evolutionarily diverged. As a result, Omp85 can selectively exclude not only non-OMPs, but also OMPs from other sources as it goes about its business of building beta barrels.

Robert V, Volokhina EB, Senf F, Bos MP, Van Gelder P, et al. (2006) Assembly factor Omp85 recognizes its outer membrane protein substrates by a species-specific C-terminal motif. DOI: 10.1371/journal.pbio.0030377

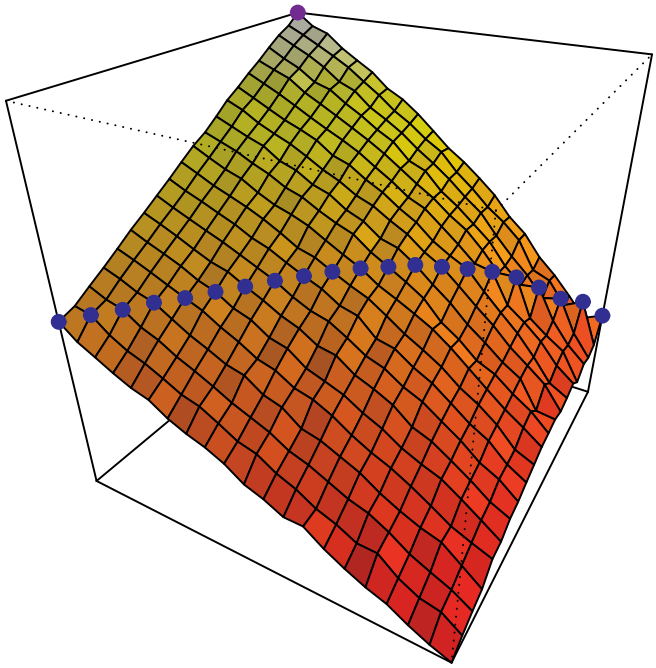
Modeling Alien Invasions: Plasticity May Hold the Key to Prevention

Liza Gross | DOI: 10.1371/journal.pbio.0040411

The fossil record shows that plant and animal extinctions have always been part of life. But today, species are disappearing at an unprecedented rate, unable to keep pace with habitat loss and alien species invasions. Exotic invasive species can quickly displace indigenous species and disrupt ecological relationships that evolved over millions of years. Invasions often alter food sources or introduce novel competitors or predators, requiring that a species modify corresponding traits (related to physiology, life history, or behavior, for example) to survive in the transfigured landscape.

In a new study, Scott Peacor, Mercedes Pascual, and colleagues derive a theory to probe the factors underlying a successful invasion. Their model included three basic elements: competition between two species, a variable environment, and a "plastic" trait that undergoes adaptive changes in response to the shifting environment. The authors hypothesized that when a flexible, adaptive response to environmental variation (called phenotypic plasticity) increases fitness, it should enhance a species' ability to invade and displace other species, once established. This fitness-related plasticity may explain why some exotic species become invasive and others don't. As expected, phenotypic plasticity exerted a "profound effect" on alien invasions, with plastic species successfully invading or resisting against invasion by an inflexible opponent. But plasticity, the authors were surprised to discover, also dramatically reduced invasion when exhibited by both invader and resident, suggesting that phenotypic plasticity can affect invasion in an unforeseen manner, independently of the fitness advantage it provides over species without plasticity.

Peacor et al. modeled the invasion of a hypothetical food chain—with a predator, resident consumer, and food source—by an invading consumer. The model assumes random environmental fluctuations and different evolutionary histories for resident and invasive species, placing the invader at the disadvantage in a foreign environment. And though higher foraging effort affords higher reproductive potential, it also risks higher predation, for both resident and alien consumers (echoing real-life risks between energy gain and death). Adding or removing the predator provides the environmental variation, and variable predation risk induces a behavioral response in prey. Both types of consumers could either discern the presence or absence of a predator and evolve bimodal foraging behavior



DOI: 10.1371/journal.pbio.0040411.g001

Phenotypic plasticity can increase the steepness of the fitness surface, which magnifies competitive differences and thereby acts as a barrier to species invasion.

(the plastic phenotype) or were unresponsive and evolved one optimal behavior for both circumstances (the nonplastic phenotype). Invasion success was measured as the time to displace the resident consumer.

When the model was run sans invasive species, plastic consumers almost always ate in the absence of predators and almost never in their presence. Nonplastic consumers, in contrast, evolved an intermediate strategy in which the probability of eating was the same (about 45%) in the presence or absence of a predator. When both resident and invader were nonplastic and had no competitive advantage (that is, the same probability of death), the invader replaced the resident. And when only the resident or invader had plasticity-enhanced fitness, the plastic resident successfully repelled the inflexible invader, and the plastic invader displaced the inflexible resident. But to the authors' surprise, invasion was rapid when both consumers were nonplastic—

yet did not occur when both consumers were plastic; plasticity effectively acted as a barrier to invasion unless invaders were given a huge competitive advantage (a 40% lower chance of death).

To understand this puzzling pattern, the authors constructed a "fitness surface," a graph plotting fitness as a function of the consumer's foraging strategy (the probability of eating in the presence or absence of the predator). Peaks on this fitness landscape correspond to adaptive traits that increase fitness and valleys to those that decrease it. Plastic and nonplastic (whether resident or invading) consumers evolved optimal behavioral strategies that corresponded to quite different fitness surfaces—the graphs reflected their respective either/or (represented by a steep slope) and "average" (plateau, then decline) optimum foraging behaviors.

Since invaders had not undergone selection in the new environment, deviating from their foraging optimum could place them at a competitive fitness disadvantage. When both consumers were nonplastic, the alien incurred only minor fitness costs by deviating from the optimum, allowing it to eventually gain a foothold. But when both consumers had plasticity, the resident's fitness landscape proved too steep to scale: when the invader strayed from its optimal strategy, it could no longer compete with the native, and died before reproducing—aborting the invasive process.

This model suggests that plasticity exerts a major influence on invasion by magnifying how even small differences in traits affect fitness. It also sheds light on natural invasive processes like colonization and vegetative succession—when new plant communities sequentially repopulate a landscape following fire, avalanche, or other disturbance—explaining how a vital community can spring from the ruins. The results also have implications for understanding species survival in fragmented landscapes, in which metapopulations persist by invading new habitat patches even as they go extinct in others.

Peacor SD, Allesina S, Riolo RL, Pascual M (2006) Phenotypic plasticity opposes species invasions by altering fitness surface. DOI: 10.1371/journal.pbio.0040372

Charting the Spread of *Salmonella* Infection

Liza Gross | DOI: 10.1371/journal.pbio.0040378

Every summer, local newspapers warn readers not to eat unchilled potato salad, seared hamburgers, and other picnic fare likely to precipitate an unpleasant encounter with *Salmonella enterica* bacteria. Yet in recent years, the number and severity of *S. enterica* cases has risen along with the number of factory farms (where infection can rapidly spread among tens and hundreds of thousands of animals) and the evolution of multi-drug–resistant *Salmonella* strains. Effective vaccine development and drug therapies depend on understanding how these pathogens behave inside the cell, but technical difficulties have limited scientists' efforts to directly observe the dynamics of infection in living tissue.

In a new study, Sam Brown, Stephen Cornell, Pietro Mastroeni, and colleagues combined microscopy and dynamical modeling techniques to identify the key variables underlying infection. Their model describes pathogen proliferation at the single cell and tissue level, producing novel insights into the dynamics of infection—and providing a framework for testing antibiotics and managing antibiotic resistance.

S. enterica pathogens initially replicate inside phagocytic immune cells; they then escape and infect other phagocytes after bursting, or lysing, the host cell. It's unclear what mechanisms induce lysis—programmed cell death or pathogenic poisons—or how they facilitate transmission to uninfected cells in a living organism.

In previous work, the authors imaged individual *S. enterica* bacteria within mouse liver phagocytes. They found that the number of infected cells increased along with the overall numbers of bacteria and that each infected phagocyte typically had low bacterial counts. Though bacterial growth rates differed—with virulent strains replicating faster than "attenuated" mutant strains—the bacterial distribution across cells remained near-constant, regardless of overall bacterial growth rate and time since infection. This observation raises the possibility that intracellular variations in bacterial counts result from inherent variations in phagocytes' response to bacterial replication.

In this study, the authors used mathematical modeling to explore possible explanations for the observed distributions and spread of infection. Proliferation dynamics within and among cells was first captured in a simple model governed by two parameters: a constant bacterial division rate—so that bacterial ancestors and all descendants reproduce stochastically, with the same probability—and host cell burst size—in which the cell bursts when bacterial numbers reach a fixed value. The model assumes that when a cell bursts, each released bacteria infects a new cell. The modeling results found that many cells had just one bacterium while others had several—as they did in the mouse phagocytes—showing that heterogeneous distributions can arise from a homogenous cell population.

Still, cells could respond differently to infection, either due to intrinsic or stochastic differences among cells. The authors modeled the effects of intrinsic variations in cellular response to infection, resulting either from different replication rates or from different burst thresholds. Variable bacterial division didn't improve the model's fit to the phagocyte data, but variable burst threshold did, predicting that cells with virulent strains would burst at much higher bacterial densities than those with attenuated strains.

The authors then considered stochastic variations among intrinsically identical cells. Assuming that bursts occur randomly rather than at a fixed threshold, they found that reducing the rate of bacterial division reduced the rate of population growth and skewed the distribution so that host

cells had fewer bacteria, like phagocytes with attenuated strains. When the authors allowed the rates of both burst and bacterial division to depend on bacterial density, the model that described their data better than all the others combined density-independent lysis with density-dependent bacterial division. This model predicts that the intracellular replication rate of *S. enterica* will decrease as the number of intracellular bacteria increases and that the bursting rate will proceed at a constant rate, with random variations, regardless of intracellular bacterial density. As before, virulent and attenuated strains behaved differently—bacterial density had a greater effect on the intracellular division rates of virulent strains.

With these insights into intra- and intercellular dynamics, the authors explored the effects of medical intervention. Since some antibiotics kill bacteria living outside cells, they refined the model to account for extracellular survival. The model showed that reducing the rate of intracellular bacterial replication leads to a reduced burst size—suggesting that the effectiveness of extracellular antibiotics would be increased if combined with antibiotics aimed at reducing intracellular replication.

These insights into the dynamic relationship between bacterial division rate, host cell lysis, and extracellular survival—and thus the probability of infecting new cells—offers a novel way of evaluating the efficacy of multi-drug therapies and the evolution of drug resistance. For example, researchers can test whether administering intracellular and extracellular antibiotics simultaneously produces synergistic effects that clear an infection. The authors hope that other researchers will incorporate new experimental results into a similar dynamical modeling approach—linking within- and among-cell microbe behavior to host response—to shed light on a wide range of host-pathogen interactions.

Brown SP, Cornell SJ, Sheppard M, Grant AJ, Maskell DJ, et al. (2006) Intracellular demography and the dynamics of *Salmonella enterica* infections. DOI: 10.1371/journal.pbio.0040349

Sexual Selection Comes at a Cost

Liza Gross | DOI: 10.1371/journal.pbio.0040394

Males evolved extravagant plumage, towering antlers, and frenetic mating displays, Darwin proposed, because that's what females like. Why such preferences evolved is still controversial—one view holds that flashy males beget sexy sons with more chance of attracting mates—but it's generally thought that sexual selection provides some type of indirect genetic benefit leading to higher intrinsic fitness of the offspring. Selection on secondary sexual characteristics often results in sexually dimorphic traits being tailored toward the specific reproductive needs of each sex. Sexual dimorphism typically arises because selection operates in different directions on each gender—selecting for large males and compact females, for example—promoting sex-specific gene expression.

But when selection acts on a shared trait and the sexes are genetically constrained from becoming dimorphic, "intralocus" sexual conflict can occur. Theoretical studies predict that sexually antagonistic genes—which favor one sex to the detriment of the other—should reduce any indirect benefits of sexual selection on high-fitness parents by compromising the fitness of opposite-sex offspring. Whether this effect is short-lived, perhaps mediated by mechanisms that restrict gene expression to the favored sex, or persists as a cost of sexual reproduction is unclear.

In a new study, Alison Pischedda and Adam Chippindale explore the potential costs of intralocus sexual conflict in the genetically tractable fruit fly, *Drosophila melanogaster*. By measuring the inheritance of fitness across generations, and across the genome, they show that sexual selection provides no advantage to the next generation. To the contrary, having a fit parent of the opposite sex leads to dramatically lower rates of reproductive success. Sexually antagonistic genes, it appears, may have far-reaching effects on patterns of fitness inheritance.

Using a recently developed genetic tool called hemiclonal analysis, researchers can screen the (nearly) entire genome

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Sexually antagonistic genes create a tug-of-war over the genome. (Image: Adam Chippindale and Helene Van)

for genetic variation within a population and for evidence of selection acting on that variation. By manipulating chromosomal inheritance in males, hemiclonal analysis extracts, clones, and amplifies male haplotypes—single sets of the three major fruit fly chromosomes, the X chromosome, and two autosomal chromosomes—from a base population to create multiple identical haploid (single copy) genomes. These genomes, considered the functional equivalent of sperm clones, are then used to fertilize many different eggs from the original base population to create individual "hemiclones" with the same haplotype expressed against a random genetic background. With this approach, it's possible to measure additive genetic variation in both female and male offspring and to estimate any selection acting on this variation, manifested as different fitness levels.

Pischedda and Chippindale used hemiclonal analysis to generate high- and low-fitness parents, and selected three lines of the most and least fit mothers and fathers, based on egg production and number of offspring sired. High-fitness females laid 35% more eggs than low-fitness females; high-fitness males fathered 44% more offspring than their less-fit counterparts. After crossing every possible combination of high- and low-fitness parental lines (yielding 36 crosses), the authors evaluated fitness effects on offspring to determine

patterns of fitness inheritance, using reproductive success of sons and daughters as measures of their fitness.

Overall, they found an inverted pattern of fitness inheritance: high maternal fitness was good for daughters but not sons, and sons born of high-fitness mothers had substantially fewer offspring than those with low-fitness mothers. Similarly, daughters sired by high-fitness fathers laid fewer eggs than those with low-fitness fathers. Paternal fitness had little effect on sons' fitness—supporting the notion that sexually antagonistic genes mostly inhabit the X chromosome, which only females transmit to sons. Thus, females that choose successful mates, the authors explain, won't see indirect benefits through sons, and to make matters worse, will incur the cost of less-fit daughters. This sexually antagonistic pattern challenges sexual selection theory predictions that female costs of reproduction are offset by the indirect benefits of passing on good genes or generating sexy sons with high reproductive success.

Many genes shaping sexual characteristics are likely affected by the conditions that favor intralocus sexual conflict in sexually reproducing organisms, the authors argue, suggesting that the phenomenon may operate in far more organisms than the fruit fly, where it was first discovered. And because sexually antagonistic genes compromise fitness by reducing fertility, the authors suggest, they may offer clues to a longstanding puzzle: how can genetic variation for a trait persist in a population in spite of strong selection in favor of one variant? Part of the answer may lie within the X chromosome: it may harbor sexually antagonistic genes that undermine offspring fitness of one sex, despite being selected for in the other sex. For now, the assembled research suggests that sexually antagonistic genes are common and consequential in the genome and powerful enough to create a reversed inheritance of Darwinian fitness across the sexes. Simply seeking out the most attractive mate may have surprising implications for the offspring.

Pischedda A, Chippindale AK (2006) Intralocus sexual conflict diminishes the benefits of sexual selection. DOI: 10.1371/journal.pbio.0040356

"Supporting" Players Take the Lead in Protecting the Overstimulated Brain

Liza Gross | DOI: 10.1371/journal.pbio.0040371

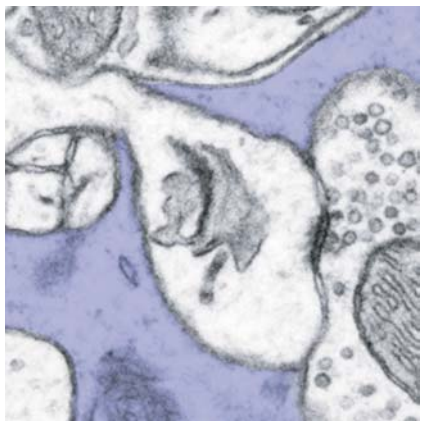
For many years, astrocytes got no respect. These star-shaped cells were long considered mere space fillers, providing structural support to buttress their betters. It's now clear that astrocytes play an active role in brain function. With their octopus-like protrusions, called processes, astrocytes remove neurotransmitters from neuron synapses, regulate the chemical composition of the extracellular environment, and can influence neuronal activity. And now a new study by Christel Genoud, Graham Knott, and colleagues provides further evidence that astrocytes take a proactive role in brain function, by showing that alterations in cortical activity cause changes in the physical interactions between astrocytes and neurons. These changes could facilitate the uptake of potentially damaging excess neurotransmitters.

Brain signals travel down the axon of a transmitting, or "presynaptic," neuron as an electrical impulse. The electrical signal is converted into a chemical signal (neurotransmitter) when the impulse reaches the presynaptic nerve terminal (or bouton). Neurotransmitters carry the signal across the gap between neurons, called the synaptic cleft, to a dendrite of the receiving, "postsynaptic" neuron. Interactions between the bouton and specialized postsynaptic protrusions in dendrites, called dendritic spines, mediate synaptic transmission.

Clearing glutamate from the synaptic cleft may be astrocytes' most critical function. The principal neurotransmitter in the brain, glutamate plays a major role in sensory perception. But too much glutamate causes trouble: as an excitatory neurotransmitter, it can stimulate the postsynaptic neuron until it is reabsorbed by membrane-bound transporters in the transmitting neuron or by transporters in astrocytes. Overstimulation can damage the nervous system, causing seizures and even stroke.

Astrocytes take up glutamate with two transporters—glutamate transporter 1 (GLT1) and glutamate aspartate transporter (GLAST)—and corral excess glutamate with their abundant processes. (Neuronal transporters recycle neurotransmitters for later use.) Genoud et al. wondered whether increased neuronal activity in the sensory cortex would trigger a corresponding response in astrocytes.

To find out, the authors hyperstimulated a single whisker in unanesthetized adult mice, a protocol that triggers physiological and morphological changes in the region of the sensory cortex that receives information from the stimulated whisker. Adult mice received 24 hours of continuous whisker stimulation The authors then located and removed the corresponding region of the cortex. Tissue was extracted immediately after stimulation from one group and four days later from another stimulated group.



DOI: 10.1371/journal.pbio.0040371.g001

Electron micrograph of a dendritic spine synapsing with an axonal bouton and flanked on either side by an astrocytic process (blue).

After 24 hours of whisker stimulation, levels of both astrocyte glutamate transporters, GLT1 and GLAST, more than doubled. Four days after stimulation had stopped, transporter levels returned to those seen in the unstimulated controls. The researchers also analyzed levels of the neuronal glutamate transporter EAAC1

in a separate group of animals but found that stimulation had little effect on its expression.

Using electron microscopy and serial sections of the stimulated brain samples, the authors used high-resolution images to generate 3D reconstructions of the contacts between astrocytes and adjacent bouton-dendritic spine pairs. These reconstructions showed that stimulation caused a marked increase in the percentage of spines with their excitatory synapses "completely occupied" by astrocytes. Although stimulation does not increase contact between dendritic spine and bouton, it does rouse astrocytes to encircle the interacting synapses, where glutamate is transmitted.

Both stimulation-triggered changes in astrocyte shape and behavior—glomming onto excitatory synapses and upregulating their glutamate transporters—help clear glutamate from the synaptic cleft. Through flexible, dynamic interactions with neurons, the authors conclude, astrocytes prevent the accumulation of glutamate, which is most likely to occur during sensory overstimulation.

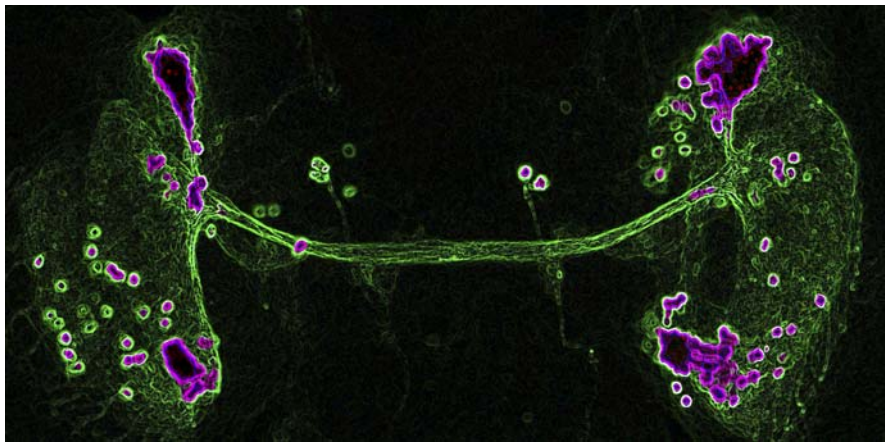
This study demonstrates yet another functional benefit of astrocyte-neuron interactions, but the authors expect to find more. Given astrocytes' control over synaptic transmission and evidence that they can release their own neurotransmitters, it seems these long-misunderstood cells are finally getting their just desserts.

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, et al. (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. DOI: 10.1371/journal.pbio.0040343

Signals That Tell Fly Neurons to Extend or Retract

Françoise Chanut | DOI: 10.1371/journal.pbio.0040391

During metamorphosis, fruit fly larvae shed the maggot sheath that had confined their life to a tedious creepy-crawl and don legs, wings, and a pair of big, buggy eyes to explore the third dimension and broaden their horizons. A less conspicuous, but just as wondrous transformation also takes place in their brains. There, new neurons are born and new neural networks are established, which the insects will use to make sense of the environment sampled by their shiny new limbs. How neurons select the appropriate partners among myriad candidates is a long-standing question in neurobiology. And what powers the growth of their axons, the long extensions with which they probe the brain until they reach the right target, is also mostly unknown. Mohammed Srahna, Bassem Hassan, and their colleagues have tackled these issues by genetically manipulating a small cluster of neurons whose axons carve their way through the optic lobe of the fly brain during metamorphosis.



DOI: 10.1371/journal.pbio.0040391.g001

The dorsal cluster neurons (magenta) and their neurites (green) forming a commissure (a bridgeway) across the adult fly brain and branching bilaterally over the fly visual centers. (Image: Lee Fradkin, Jasprien Noordermeer, and Bassem Hassan)

The neurons, known as dorsal cluster neurons (DCNs), lie in clusters of approximately 40 near the top of each brain hemisphere. At the end of larval life, they start extending a long axonal branch across the brain, all the way to the opposite optic lobe (contralateral branch). Using a gene construct specifically expressed in the DCN *(ato-Gal4-14a)*, the researchers marked the neurons with a fluorescent protein to follow their axons' trajectories. A third of the way through metamorphosis, most contralateral branches (30–40) had passed the lobula and reached the medulla, two neighboring brain areas that integrate neuronal inputs coming from the retina. By the adult stage, however, only a dozen contralateral branches remain connected to the medulla. The researchers suspected that the other branches degenerated in the late phases of metamorphosis. Indeed, they observed shortened DCN axons between lobula and medulla in flies mid-way through metamorphosis. But they found no evidence of axon breakdown, such as swelling or fragmentation. They concluded that most DCN axons retracted back to the lobula after reaching the medulla.

To probe the mechanics of DCN axon growth and retraction, the researchers blocked specific signaling pathways in the DCN by driving the expression of various gene constructs with *ato-Gal4-14a*. Blocking the intracellular signaling protein JNK or a receptor (Fz2) for the extracellular signal Wnt prevented the extension of DCN axons beyond the lobula. Blocking another intracellular signaling protein called Rac1 or the receptor for the extracellular signal FGF caused more DCN axons than normal to innervate the medulla. By simultaneously manipulating several pathways, the researchers determined how the pathways interact to regulate DCN axon growth. For instance, blocking JNK suppressed the excessive medulla innervation caused by blocking Rac1. This observation suggests that JNK is necessary for DCN axons to reach the medulla and that Rac1 normally impairs the activity of JNK.

Based on additional genetic and biochemical analyses, the researchers

propose that JNK signaling is the engine that drives the growth of the DCN axons all the way to the medulla. In the medulla, the axons encounter a localized pool of FGF, which causes some of them to retract back to the lobula. FGF signaling acts by facilitating the inhibition of JNK by Rac1 within the DCN. In contrast, Wnt signaling boosts the activity of JNK by blunting the inhibitory effect of Rac1. The researchers propose that Wnt signaling in DCNs allows axons to maintain their connection to the medulla. Extension or retraction of DCN axons might therefore reflect a different balance of the antagonistic effects of FGF/Rac1 and Wnt/JNK signaling. Single-cell mutations suggest that the integration of these signals is happening in each of the 40 DCNs independently, meaning that the global pattern arises as a result of the autonomous action of individual cells. What remains now to be understood is what makes the 12 or so DCNs that stay connected to the medulla strike that balance differently from their 28 counterparts.

Srahna M, Leyssen M, Choi CM, Fradkin LG, Noordermeer JN, et al. (2006) A signaling network for patterning of neuronal connectivity in the *Drosophila* brain. DOI: 10.1371/journal.pbio.0040348

The Seeds of Diversity: Lessons from Tropical Trees

Liza Gross | DOI: 10.1371/journal.pbio.0040375

Understanding the mechanisms that support biodiversity has long been a fundamental problem in ecology. But with species disappearing roughly 1,000 times faster than they did before humans entered the picture, the question is hardly academic.

As biodiversity hotspots, tropical jungles provide a fertile ground for testing theoretical predictions about what drives diversity. Tropical trees of the same species (called conspecifics) often cluster in scales ranging from a few meters to a few hundred meters. Theoretical studies explain why clustering may promote diversity—by separating species and thus reducing competition between them—but evidence supporting different views of what causes clustering has been limited. Studies have established that limited seed dispersal of tropical pioneer trees (the first to colonize a disturbed landscape) in turn limits the spatial distribution of their seeds and seedlings. But without evidence that limited dispersal also affects the spatial distribution of mature trees, the notion that dispersal underlies community structure and biodiversity remains hypothetical.

In a new study, Tristram Seidler and Joshua Plotkin provide that evidence by comparing the dispersal mechanisms and spatial distribution of 561 tropical tree species in a forest reserve in peninsular Malaysia. By demonstrating a strong correlation between the degree of conspecific clustering and the mechanism of dispersal, they show that dispersal characteristics have long-lasting effects on community structure.

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Tree trunks and vines at the Pasoh Forest Reserve, one of the last remaining examples of primary lowland tropical forest in peninsular Malaysia. (Photo: T. Seidler)

Instead of waiting decades for seedlings to mature so they could determine how seed fall affects the spatial distribution of mature trees, Seidler and Plotkin exploited the diversity of dispersal mechanisms across a broad range of species to investigate the relationship. Of 637 tree species within a 50-hectare area, the authors were able to assign dispersal mechanisms to 561 species—based on field data, specimens, and published descriptions of fruit anatomy and morphology. Dispersal mechanisms included ballistic (often described as the "explosive liberation" of seeds), gravity, gyration, wind, and three animal categories based on fruit size. (Smaller passerine songbirds and perching birds, for example, tend to eat and disperse small fruits, while larger birds and mammals tend toward larger fruits.)

To detect patterns in the observed distribution of trees in the study plot, Seidler and Plotkin quantified the degree of spatial aggregation for each species. Based on the observed distribution of conspecific trees, they computed the average spatial cluster size for each species. They found a strong relationship between the cluster size of a species and its dispersal mechanism. Ballistic dispersal produced the most aggregated clusters, followed by gravity, gyration, wind, and animal. (Small-fruited tree species were the most aggregated, and large-fruited trees were the most widely distributed.) These correlations held up after accounting for confounding factors that could arise as a result of evolutionary kinship among species.

To paint a more detailed picture of the relationship between dispersal and spatial pattern, Seidler and Plotkin also quantified aggregation for each species over a range of spatial scales. The relationship between clustering and dispersal mechanism was strongest at smaller scales, while at larger scales (over 200 meters), aggregation did not vary among dispersal mechanisms. The authors found similar patterns in a Panamanian jungle with a markedly different community structure, suggesting that the relationship between dispersal and distribution is not unique to their Malaysian study plot.

These results support several theoretical predictions about the ecological attributes of seed dispersal among tropical trees. The finding that animal-dispersed, small-fruit–bearing trees aggregate more than those producing large fruit supports

the hypothesis that larger birds and mammals eat bigger fruits, roam larger territories, and transport seeds farther than smaller animals do. And the tight clustering associated with wind-dispersed species likely results from the forest canopy's constraining effects on wind currents. The authors caution that their dispersal-mechanism categories are oversimplified and that other factors influence how conspecific trees take root in a particular community. But their results provide direct evidence for theories of how forest communities develop and how variation in dispersal mode promotes diversity—valuable insights for a planet that is losing, or expected to lose, an estimated 0.25% of tropical forest species every year.

Seidler TG, Plotkin JB (2006) Seed dispersal and spatial pattern in tropical trees. DOI: 10.1371/journal.pbio.0040344

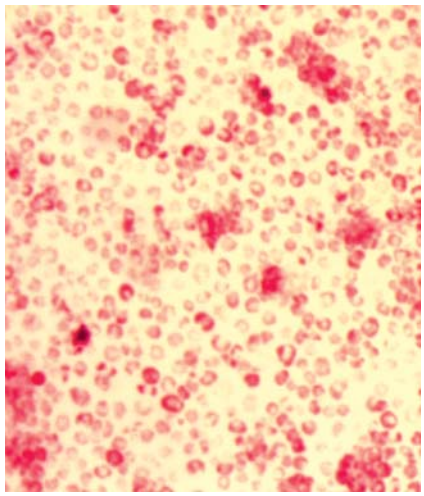
HIV–Cholesterol Connection Suggests a New Antiretroviral Strategy

Liza Gross | DOI: 10.1371/journal.pbio.0040400

Straddling the line between living and nonliving, a virus must commandeer the molecular machinery of the cell it infects to persist, and sometimes even alters the cellular environment toward that end. The HIV genome encodes nine proteins, some playing multiple roles to help the virus invade human immune cells and co-opt cellular proteins—and even normal cellular activities—to reproduce. For example, a recent study showed that an HIV protein called Nef, which is required for viral replication and infection, also binds cholesterol and delivers it to the cell membrane where new virus particles (called virions) are assembled. Once assembled, virions take part of the lipid-rich cell membrane with them as they bud off in search of new cells to infect.

Now, in a new study, Zahedi Mujawar, Michael Bukrinksy, Dmitri Sviridov, and colleagues show that the HIV Nef also disrupts cholesterol trafficking machinery in the macrophages infected by the virus. By preventing the normal efflux of cholesterol from macrophages, Nef ensures that nascent virions have access to a steady supply of cholesterol. Unfortunately, what's good for the virus causes even more trouble for a person infected with HIV: reduced cholesterol efflux from macrophages may explain why patients with HIV face increased risk of atherosclerosis and coronary artery disease.

Normally, excess cholesterol is exported to molecules in the extracellular space. The authors found that cholesterol efflux to an extracellular molecule called apoA-I was impaired in HIV-infected macrophages. Lower levels of efflux correlated with higher levels of viral replication. To see whether Nef might affect efflux impairment, the authors infected macrophages with HIV strains carrying either a functional or mutated *Nef* gene (known to affect cholesterol binding and delivery). Cholesterol efflux to apoA-I was "substantially reduced" in cells infected with functional Nef. In contrast, cholesterol efflux in cells infected with Nef-deficient strains was similar to that seen in uninfected cells—allowing the authors to conclude that HIV needs Nef to block cholesterol efflux.



DOI: 10.1371/journal.pbio.0040400.g001

HIV-infected monocyte-derived macrophages show lipid accumulation (lipids are stained red) in HIV-infected cells. (Image: Zahedi Mujawar)

Cholesterol is removed from macrophages by proteins called ATP-binding cassette (ABC) transporters, which hand off their cargo to different molecules. Having shown that Nef hinders efflux to apoA-I, the authors reasoned that Nef does so by targeting apoA-I's donor—ABCA1. This conclusion is supported by two lines of evidence: stimulating expression of ABCA1 in infected cells significantly reduced Nef-mediated impairment of efflux to ApoA-I, but efflux was unaffected when ABCA1 levels were very low. Nef mediates these effects, the authors show, by specifically down-regulating ABCA1 after transcription and limiting its abundance, and by keeping ABCA1 sequestered at the membrane. Whether these mechanisms work in tandem or operate independently is a question for future study.

HIV-infected macrophages that expressed Nef not only contained far more lipid-containing vacuoles than uninfected cells or cells infected with Nef-deficient HIV, they also synthesized molecules associated with cholesterol accumulation and cholesterol-laden "foam cells." And when the authors analyzed atherosclerotic plaque sections taken from patients with HIV who had been treated with anti-retroviral therapy (HAART), they found HIV-infected, cholesterol-laden macrophages in the atherosclerotic plaque—suggesting that these cells contribute to arterial disease.

Altogether, the results show that HIV inhibits cholesterol efflux through Nef, leading to cholesterol accumulation and foam cell formation. They also suggest potential targets for controlling both HIV and atherosclerosis. When the authors increased ABCA1 levels to stimulate cholesterol efflux—having observed a link between reduced efflux and increased viral replication—the treatment restored efflux and reduced virion infectivity by reducing virion incorporation of cholesterol. This suggests that stimulating cholesterol efflux may not only reduce the risk of atherosclerosis but may also help control HIV replication in HIV-infected patients.

Mujawar Z, Rose H, Morrow MP, Pushkarsky T, Dubrovsky L, et al. (2006) Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. DOI: 10.1371/journal.pbio.0040365

Assessing Ecosystem Services to Identify Conservation Priorities

Liza Gross | DOI: 10.1371/journal.pbio.0040392

Efforts to save wilderness often play out within a win-lose framework, pitting conservation against economic opportunity. But as human pressures on wildlands continue to escalate, conservation biologists are seeking win-win approaches, based on the notion that ecosystems provide numerous economic benefits—wetlands mitigate flooding, for example—to a wide variety of beneficiaries. By quantifying these ecosystem services and the "opportunity" costs of not developing habitat, planners can identify areas that provide important ecosystem services and determine who benefits from these services and who incurs costs. But quantifying costs and benefits and the flow of ecosystem services across a variable landscape is a daunting task. Thus far, it has not been clear to what degree traditional conservation plans for biodiversity also protect valuable ecosystem services.

Taking complementary approaches to this problem, two new studies use spatially explicit models to incorporate ecosystem services into conservation planning. In one study, Robin Naidoo and Taylor Ricketts weigh the economic value of five ecosystem services against the costs of conservation in the Atlantic forests of Paraguay. In the second study, Kai Chan, Rebecca Shaw, Gretchen Daily, and colleagues present a strategy for integrating ecosystem services into biodiversity conservation plans in California's Central Coast ecoregion to systematically identify priorities for conservation.

Naidoo and Ricketts assessed five ecosystem services—sustainable bushmeat harvest, sustainable timber harvest, pharmaceutical bioprospecting, existence value (the intrinsic value of unspoiled wilderness), and carbon storage (forest conversion releases carbon dioxide)—provided by forests in the Mbaracayu Biosphere Reserve. The reserve—once covered by 90% forest but now highly fragmented and threatened beyond a protected core—supports large-scale cattle ranching, soybean production, and small-scale farming, along with hunting and foraging by the indigenous Ache.

In a previous study (co-authored by Naidoo), the opportunity costs of conserving forested land had been estimated by integrating expected agricultural production values with the probability of forests being converted to agriculture; the latter was calculated based on past patterns of deforestation. This procedure provided an estimate of the opportunity costs of conservation for each hectare of forest in the reserve.

To calculate conservation benefits, Naidoo and Rickets determined the beneficiaries and value of each ecosystem service per forest parcel across six forest types. There is no market price for bushmeat because it cannot be legally traded, so the authors calculated its value in part by multiplying the local price of a kilogram of store-bought beef (US$1.44) times the expected meat production (from 12 wild game species) for each forest hectare. Sixteen tree species in the reserve were used to estimate the average value of marketable timber per standing tree (US$6.87), assuming a sustainable harvest. Bioprospecting value was calculated based on drug companies' willingness to pay for potentially marketable forest-derived drugs. Existence value was estimated based on various groups' willingness to pay for forest preservation. And carbon storage value was calculated based on prices in the carbon-trading market (where companies get paid for stemming carbon dioxide emissions).

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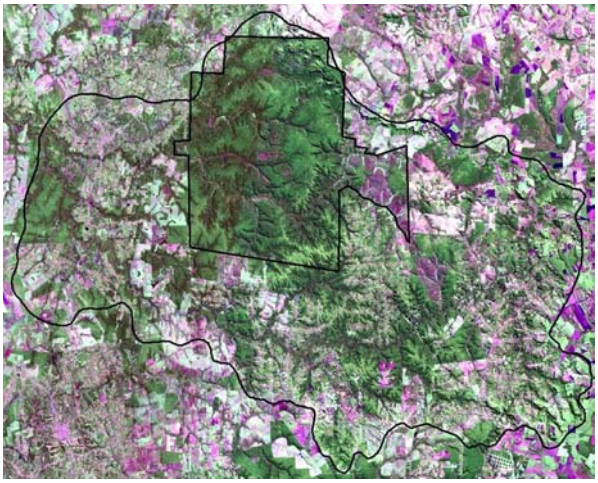
The moist forests of Point Lobos State Reserve and the northern Santa Lucia Range are important for biodiversity, carbon storage, flood control, recreation, and water provision. (Photo: Kai Chan)

Both the costs and the benefits of forest conservation varied considerably across this relatively small landscape. Interestingly, bioprospecting—long invoked to justify conserving areas that could harbor potentially life-saving medicines—offered the smallest benefit. When only local (bioprospecting, bushmeat, timber) services were included in the analyses, just a few parcels passed the cost-benefit test for conservation. But when carbon values were added—"by far" the most valuable service on average per hectare—benefits exceeded opportunity costs for 98% of the forests. Demonstrating how this approach could guide conservation planning, the authors show that linking two large forest patches with one wildlife corridor would provide far greater net benefits than either of two alternative corridors.

Building on recent advances in the emerging field of ecosystem service conservation, Chan et al. present an analytical framework that integrates ecosystem services into the well-established methods of biodiversity planning. Innovative "payment for environmental services" schemes, in which people who benefit from ecosystem services pay for them, have been introduced on a small scale in Guatemala, Indonesia, and other countries (and on a larger scale in Costa Rica). But expanding on these local programs requires a comprehensive, quantitative planning framework that links ecosystem services with biodiversity conservation. Toward that end, Chan et al. mapped the intersection between areas that provide ecosystem services and those that support substantial biodiversity. By modeling the implications of conservation plans based on prioritizing various combinations of biodiversity and ecosystem services—identifying tradeoffs in some cases and synergies in others—they illustrate the importance of integrating both elements in conservation planning.

The authors studied the California Central Coast ecoregion, covering north of San Francisco to Santa Barbara,



DOI: 10.1371/journal.pbio.0040392.g002

Landsat satellite image of the Mbaracayu Forest Biosphere Reserve. Forests (dark green) are steadily being converted to pastures, croplands, and industrial soybean farming. Economic arguments for deforestation usually ignore the important ecosystem services that such forests provide. (Image: Laura Rodriguez, Juan Viera, and Robin Naidoo)

where they had sufficient empirical and spatially explicit datasets for six ecosystem services provided by the 11,000-plus planning units in the region. They mapped terrestrial biodiversity and six services—carbon storage, flood control, forage (grazing) production, outdoor recreation, crop pollination, and water provision—across the region, and estimated each parcel's provision of each service (following the same protocol used to plan biodiversity conservation) to develop conservation area networks for each service and biodiversity.

With a conservation planning software program, they mapped the spatial correlations between benefits (biodiversity and the six services) across parcels. They also quantified ecosystem services provided by a network of lands prioritized for biodiversity versus multiple ecosystem services. The spatial distribution of benefits from biodiversity and each of the six ecosystem services varied considerably across the ecoregion. Some mountain regions, for example, had high values for carbon storage, water provision, and recreation, while the agricultural Salinas Valley had high pollination service values and a small riparian area that provided flood control but low values for other services. Overall, spatial correlations between the ecosystem services were low (aside from a few high correlations, such as between carbon storage and water provision), as was the correlation between biodiversity and the other services. The authors found that there were generally low levels of overlap between planning units prioritized for different services.

The authors also determined tradeoffs between plans based on biodiversity versus ecosystem services by analyzing four different combinations of biodiversity/ecosystem networks; they also determined potential side benefits of adding ecosystem services to a biodiversity network. When biodiversity networks were considered as a whole, they found that "impressive supplies of ecosystem services" would be protected. Plans that prioritized networks based on ecosystem services—either with or without biodiversity, or a "strategic" set, with biodiversity but without the two agriculture-based services—protected slightly higher levels of services but lower levels of biodiversity. The strategic network performed well for biodiversity and had the best overall levels of all benefits. These results reveal important potential tradeoffs between conservation for biodiversity versus ecosystem services, underscoring the need for a systematic conservation planning framework that strategically targets the unique complement of features in a given region.

Understanding the degree of overlap between lands that provide important ecosystem services as well as biodiversity, Chan et al. argue, will not only reveal hotspots for conservation but also suggest new partners for ecosystem protection. It also provides an opportunity to build common ground between wilderness advocates and landowners to develop conservation initiatives with multiple winners. By the time hurricane Katrina hit, Louisiana had lost 405,000 hectares of wetlands—offering a bitter lesson on wetlands flood protection. Now, a growing list of industries sees wetlands restoration as the key to economic recovery in the area.

Together, these two studies contribute valuable analytical frameworks to the nascent field of studying and planning for ecosystem services. And by systematically identifying tradeoffs and opportunities for aligning plans to protect biodiversity with those to conserve the flow of services from an ecosystem, they provide policymakers with a decision-making framework to identify conservation hotspots and maximize the allocation of scarce conservation dollars.

Naidoo R, Ricketts TH (2006) Mapping the economic costs and benefits of conservation. DOI: 10.1371/journal.pbio.0040360

Chan KMA, Shaw MR, Cameron DR, Underwood EC, Daily GC (2006) Conservation planning for ecosystem services. DOI: 10.1371/journal.pbio.0040379

Kinetics of Synaptic Protein Turnover Regulate Synaptic Size

Rachel Jones | DOI: 10.1371/journal.pbio.0040404

When most of us picture a cell—or any subcellular component, like the nucleus—we often imagine a fairly static, solid entity. The molecules of the membrane and all the intracellular machinery fit together like pieces of a jigsaw puzzle. But in reality, the proteins, lipids, and other molecules that make up a cell and its parts are incredibly mobile and often short-lived.

In this unstable environment, how does the cell maintain and control its various functions? Karel Svoboda and colleagues have addressed this question by investigating how a protein called PSD-95 spreads within cells and how this transport and diffusion modulate the strength and size of neuronal connections. PSD-95 inhabits a compartment in neuronal synapses (the communication junction between neuron pairs) called the postsynaptic density, where the receptors that detect neurotransmitters released by a neighboring neuron are sited. PSD-95 helps to anchor these receptors in place. In certain types of synapses, the

postsynaptic density caps the end of a specialized structure called a spine, which looks a little like a tiny mushroom sticking out from the cell membrane.

Synapses and spines can grow and shrink, and they appear and vanish throughout life, but others are stable and can last for months. However, the proteins that form essential structures in the postsynaptic density and spine, including PSD-95, last for only hours. Svoboda's team set out to investigate the dynamics of clusters of PSD-95 and how they affect spine and synapse stability.

To be able to see spines in living brains, the authors introduced the genes for two proteins—a red fluorescent protein called mCherry, and PSD-95 tagged with a green fluorescent protein (GFP)—into neurons in embryonic mice. After the mice were born, Svoboda and colleagues removed a small piece of their skulls and replaced it with a tiny "window," through which they could view the brain. Using a specialized technique called dual-laser two-photon laser scanning microscopy, they could see individual spines and the distribution of green fluorescent PSD-95. Within the spines, and particularly at their tips, green fluorescent buds (called puncta) represented clusters of PSD-95. These clusters did not seem to move, shrink, or grow over the course of a 90-minute imaging session. In some instances, these clusters were stable for days.

To investigate the behavior of individual molecules of PSD-95, the authors used a form of GFP that is normally not visible but can be "photoactivated" by a specific wavelength of light. After the photoactivation, bright fluorescence in the spines faded (over tens of minutes), showing that the photoactivated molecules of PSD-95 were leaving and, presumably, being replaced by nonphotoactivated molecules that entered the postsynaptic density from elsewhere. At the same time, fluorescence gradually appeared in neighboring spines, indicating that photoactivated PSD-95 was moving between spines. The time course of this turnover was much less than the lifetime of a spine or the half-life of PSD-95. While simple diffusion could predict how quickly PSD-95 exchanged between synapses, Svoboda and colleagues found that the rate of PSD-95 turnover within spines is mainly a function of its binding to other molecules in the postsynaptic density.

Large spines contain more PSD-95 than smaller ones and are also more stable. If the kinetics of PSD-95 at all synapses were identical, diffusion would eventually lead to all synapses containing the same amount of PSD-95. But this does not happen—larger postsynaptic densities capture more PSD-95 and retain it longer than smaller ones. Changes in size and changes in retention time are highly correlated. These synapse-specific kinetics could result from the effects of spine geometry or from biochemical mechanisms, such as differences in binding partners for PSD-95 between synapses of different sizes, or from synapse-specific regulation of post-translational modifications of PSD-95.

Synapses and spines become more stable with increasing age during development, and Svoboda's team showed that the retention of PSD-95 in the postsynaptic density increases in parallel. But synapses are also highly plastic, responding rapidly to changes in synaptic activity. When the inputs to the area of cortex being studied were reduced—by trimming off the whiskers that provide sensory input to that part of the somatosensory cortex in mice—PSD-95 retention at the postsynaptic density dropped markedly, showing that the kinetics of PSD-95 turnover are experience-dependent.

These findings show that PSD-95 is shared between synapses and spines, and that the kinetics of PSD-95 turnover within spines are tuned to maintain the appropriate size of the postsynaptic density at each synapse. Attention will now turn to the mechanisms of this tuning and to the potential role of PSD-95 in contributing to synaptic plasticity.

Gray NW, Weimer RM, Bureau I, Svoboda K (2006) Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. DOI: 10.1371/journal.pbio.0040370

Apoptosis Versus Survival: When in Doubt, Choose Both

Richard Robinson | DOI: 10.1371/journal.pbio.0040408

To function, a protein must fold up after it is synthesized. When it doesn't, trouble can ensue—the accumulation of unfolded proteins is a common cause of degenerative disorders such as Parkinson disease. When unfolded or misfolded proteins begin to build up, the cell sets in motion a coordinated cascade of events termed the "unfolded protein response" (UPR). In fact, the UPR comprises two distinct pathways leading to opposite fates: down one path lies adaptation and continued life for the cell, while down the other lies the programmed cell death known as apoptosis.

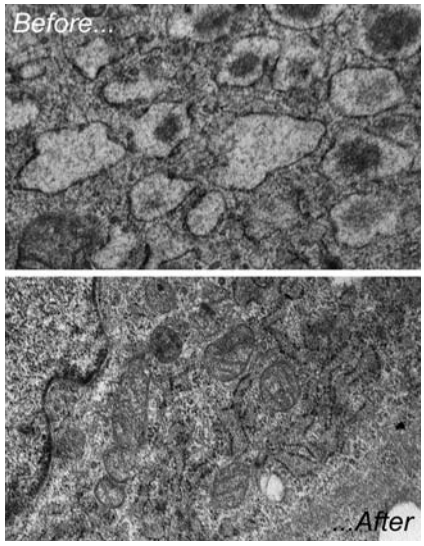
It has been known for some time that the choice of pathway depends in part on the amount of unfolded protein: small amounts lead to adaptation and large amounts to death. But how does the cell select between these two paths? In a new study, Thomas Rutkowski, Randal Kaufman, and colleagues show that even when unfolded protein levels are low, both paths are activated. But the signals that lead to apoptosis are short-lived, and in the absence of high amounts of unfolded protein, they cannot trigger further events in the apoptotic chain before they are degraded.

The authors began by treating cells in culture with either one of two chemicals that disrupt protein folding, activating the UPR. At high chemical levels, the cells died. But at low levels, the cells adapted to the stress, continuing to proliferate, largely avoiding apoptosis, and reducing the level of unfolded protein over time. In addition, once the cells had undergone adaptation, they were more resistant to further stress, either from the original stressor chemical, or from the other one of the pair.

The UPR begins when a protein called BiP, normally bound to three other proteins, instead binds increasingly to accumulating unfolded protein. Without BiP, these three proteins—called PERK, IRE1, and ATF6—become activated, which is the crucial trigger for both the adaptive and terminal responses. The authors showed that both high and low chemical concentrations activated

the three proteins, indicating that the difference in outcome was not due to a failure to start down the apoptotic pathway. However, another protein was differentially affected by chemical concentration. CHOP is a pro-apoptotic protein further down the cell-death pathway. Both high and low chemical concentrations stimulated CHOP production, but when the chemicals were at low concentration, the increase in CHOP didn't last, and the cells survived. BiP was also increased by the two chemicals, but unlike CHOP, its up-regulation persisted at low concentrations.

This difference between BiP and CHOP was not due to a difference in the amount of mRNA produced from the two genes. Instead, the authors showed, the CHOP mRNA was less stable, degrading within hours, while the BiP mRNA persisted. Thus, BiP proteins continued to be produced long after CHOP production had ceased. Similarly, the BiP protein was more stable than the CHOP protein. The excess of BiP allowed the three protein triggers—PERK, IRE1, and ATF6—to be re-bound and decommissioned, while the cell increased its production of other proteins to reduce the level of unfolded proteins that began the entire process. Only when the stressing chemicals were at high concentration did enough CHOP protein last long enough to tip the balance toward apoptosis rather than adaptation.



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Electron microscopic images of the endoplasmic reticulum in mouse embryonic fibroblasts that are either newly exposed (top) or adapted (bottom) to stress.

Finally, the authors showed that the same prolonged adaptive response can occur not only when the stress arises from chemical insult but also from a genetic defect in the protein quality control machinery. Challenged with chemical endoplasmic reticulum stress, such cells were relatively resistant to damage, demonstrating a pre-existing adaptation due to the chronic low-level stress as a consequence of the genetic mutation.

These results highlight a versatile strategy for choosing in the face of uncertainty: when in doubt, choose both. When stressed, the cell initiates both survival and death pathways. The system is weighted toward survival, but if the situation continues to be dire and apoptosis becomes the preferred result, the cell is primed for its final act. Further exploration of this system is likely to reveal more about programmed death and its alternative, and how the cell chooses between them.

Rutkowski DT, Arnold SM, Miller CN, Wu J, Li J, et al. (2006) Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. DOI: 10.1371/journal.pbio.0040374