

## Synopsis of Research Articles

## Plus Ça Change: Gene Enhancers Upset Evolutionary Assumption

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A standard evolutionary assumption is that the DNA of closely related species should be more similar in both structure and function than that of more distantly related ones. This parsimonious rule of thumb holds true across wide expanses of time and among widely divergent species, but it has exceptions. In this issue, Michael Ludwig and colleagues show that one exception is in an enhancer region of a key developmental gene in fruitflies of the genus *Drosophila*. Here, the enhancer is functionally similar enough in two species that diverged 60 million years ago that switching them produces normal development, but different enough in two species separated by only 10 million years that exchanging them between the two flies aborts development.

The gene in question is *even-skipped*, a patterning gene that creates seven transverse stripes along the anterior–posterior axis in the fruitfly embryo. Its expression is regulated by five enhancing elements located upstream from the promoter. The best characterized of these, the stripe 2 enhancer (S2E), binds five different transcription factors at multiple locations.

Ludwig et al. deleted S2E in *D. melanogaster*, and then added back S2E from one of four *Drosophila* species: *D. melanogaster* itself; *D. yakuba* or *D. erecta*, both of which have been separated from *D. melanogaster* for 10–12 million years; or *D. pseudoobscura*, which split from the *D. melanogaster* line 40–60 million years ago. Despite serving identical

functional roles in each species, the structures of these enhancers differ, with large deletions and insertions between transcription factor binding sites, as well as other changes. Nonetheless, within each species, the spatiotemporal pattern of expression induced by S2E is essentially identical, suggesting that, despite their structural differences, they might be functionally interchangeable.

Since loss of S2E is lethal, the viability of the embryos that resulted from these experiments gives a measure of each enhancer's ability to function in its new environment. The authors found that while viability of *D. melanogaster* with the *D. pseudoobscura* S2E was identical to that with *D. melanogaster*'s own, viability with S2E from the more closely related *D. erecta* was almost zero, essentially the same as not having the enhancer at all. S2E from *D. yakuba* impaired the viability of *D. melanogaster* as well, although not as much as that from *D. erecta*. Viability was closely correlated with the level of stripe 2 expression induced by each S2E, with very low levels induced by the *D. erecta* enhancer and normal levels by that of *D. melanogaster* and *D. pseudoobscura*.

Why did the *D. erecta* enhancer fail to respond in the *D. melanogaster* environment? Ludwig et al. suggest it may be due to a change in the sensitivity of the “set point” in *D. erecta*'s enhancer, which acts like an on–off switch governing gene expression, making the enhancer unresponsive to the gradients of transcription factors found in *D. melanogaster*. This change

may be due to relatively small differences in the two species' enhancers that have accumulated since their evolutionary split.

The results of this study indicate an important caveat about interpreting the evolution of gene regulatory regions. As a complex functional unit that integrates a host of signals, the S2E is likely to be under strong stabilizing selection, maintaining its output within narrow limits. Thus, the phenotypic result of the enhancer—the location and timing of stripe formation it induces in its native environment—remains conserved among the four species. However, unlike an enzyme or structural protein, in which structural changes are tightly constrained by their effects on function, the structure of any particular enhancer need not be so rigidly preserved. As long as the consequences of change in one region, such as loss of a transcription factor binding site, are matched by compensatory changes in another, such as gain of one, or, as Ludwig et al. speculate, by complementary changes in genetic background, the final output of the enhancer can remain the same. Thus, the utility of structural similarities in understanding evolutionary relationships is likely to be less for gene regulatory regions than for structural genes or the proteins they encode.

Ludwig MZ, Palsson A, Alekseeva E, Bergman CM, Nathan J, et al. (2005) Functional evolution of a cis-regulatory module. DOI: 10.1371/journal.pbio.0030093

## Gray Wolves Help Scavengers Ride Out Climate Change

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Average earth temperatures rose 0.6 °C over the last century, according to the latest Intergovernmental Panel on Climate Change. But that increase pales in comparison to the 1.4–5.8 °C expected increase over this century. As temperatures climb, climate models predict that high-latitude, high-altitude regions like Yellowstone National Park will experience shorter winters and earlier snow melts. How these environmental shifts will impact species and ecosystems remains to be seen.

The effects of climate change are already evident at the species level, with disruptions in range, reproductive success, and seasonal phenomena like migration, and the decoupling of evolutionarily paired events like new births and food availability. Both experimental and data-driven modeling studies predict that climate change may well precipitate shifts in the structure of ecosystems as well.

In a new study, Christopher Wilmers and Wayne Getz investigated the

effects of climate change on ecosystem dynamics by studying a keystone species in Yellowstone, the gray wolf (*Canis lupus*). Gray wolves inhabited most of North America until US extirpation campaigns nearly eradicated them by the 1930s. In 1995, the US Fish and Wildlife Service reintroduced the persecuted predator into Yellowstone.

Wilmers and Getz used data from the past 50 years at two weather stations in the park's northern range (where elk over winter and four to six wolf packs now

live) to establish winter trends and model wolves' impact on the fate of resident scavengers faced with a changing climate. Not surprisingly, their models show that this top predator exerts significant influence over animals at lower levels in the food chain: wolf kills temper the potentially devastating effects of climate-related carrion shortages on scavengers. Unlike mountain lions and grizzly bears, wolves abandon their prey (usually elk or moose) once sated, leaving much-coveted leftovers for ravens, eagles, coyotes, bears, and other scavengers. These findings indicate that individual species stand a better chance of adapting to climate change in an ecosystem with an intact food chain.

Wilmers and Getz's weather data analysis found that both late-winter snow depth and snow-cover duration have decreased significantly since 1948—winters in Yellowstone are getting shorter. That's good news for elk—navigating deep snow taxes stamina and reduces access to forage—but bad news for scavengers that rely on elk carcasses to carry them through the winter.

The authors generated two sets of models to estimate the effects of shorter winters on the wolf–elk–scavenger dynamics. In the first, late-winter carrion availability drops by 66% without wolves but by only 11% when the predators are present. The second model examines the impact of elk and wolf population dynamics on carrion availability. This analysis predicts that more elk will die in early winter than in late winter, a scenario that favors eagles and ravens—which can cover a lot of ground quickly—over bears and coyotes. Altogether, these modeling



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#### Reintroduced wolves do their part: an intact food chain buffers the impact of deteriorating environmental conditions (Photo: Dan Hartman)

studies show that shorter winters without wolves will create intermittent food supplies that no longer track the needs of local scavengers. With or without wolves, late-winter carrion abundance will decline with shorter winters. But wolf kills buffer these shortages, providing meals that could determine whether scavengers will be able to survive and reproduce.

It seems clear that wolves have the potential to provide a safety net for scavengers, extending the time they need to adapt to a changing environment. Thanks to a rebounding wolf population, field researchers can measure the magnitude of this predicted buffer effect. The models described here can guide their

efforts and help species adjust to major environmental shifts like climate change.

As a young US ranger “full of trigger-itch,” Aldo Leopold killed his share of wolves under the federal eradication policy—until he “watched a fierce green fire dying” in the eyes of a slain mother flush with pups and realized he had not understood the wolf’s ecological role. Wilmers and Getz’s study shows that a robust food chain—including this still embattled top predator—may be even more important as ecological conditions deteriorate.

Wilmers CC, Getz WM (2005) Gray wolves as climate change buffers in Yellowstone. DOI: 10.1371/journal.pbio.0030092

## The Chimp Genome Reveals a Retroviral Invasion in Primate Evolution

DOI: 10.1371/journal.pbio.0030126

It’s been known for a long time that only 2%–3% of human DNA codes for proteins. Much of the rest of our genomes—often referred to as junk DNA—consists of retroelements: genomic elements that are transcribed into RNA, reverse-transcribed into DNA, and then reinserted into a new spot in the genome. Human endogenous retroviruses make up one class of these retroelements. Retroviruses can insinuate themselves into the host’s DNA in either the soma (nonreproductive cells) or the germline (sperm or egg).

If the virus invades a nonreproductive cell, infection may spread, but viral

DNA will die with the host. A retrovirus is called endogenous when it invades the germline and gets passed on to offspring. Because endogenous retroviruses can alter gene function and genome structure, they can influence the evolution of their host species. Over 8% of our genome is made of these infectious remnants—infections that scientists believe occurred before Old World and New World monkeys diverged (25–35 million years ago).

In a new study, Evan Eichler and colleagues scanned finished chimpanzee genome sequence for endogenous retroviral elements, and found one

(called PTERV1) that does not occur in humans. Searching the genomes of a subset of apes and monkeys revealed that the retrovirus had integrated into the germline of African great apes and Old World monkeys—but did not infect humans and Asian apes (orangutan, siamang, and gibbon). This undermines the notion that an ancient infection invaded an ancestral primate lineage, since great apes (including humans) share a common ancestor with Old World monkeys.

Eichler and colleagues found over 100 copies of PTERV1 in each African ape (chimpanzee and gorilla) and Old World



## And Littler Genomes inside 'Em: Clues to a Parasitic Nematode's Bacterial Partnership

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"Great fleas have little fleas upon their backs, to bite 'em. And little fleas have lesser fleas and so on, ad infinitum."—Augustus DeMorgan, based on a Jonathan Swift poem

More than a billion people are at risk for infection with filarial nematodes, parasites that cause elephantiasis, African river blindness, and other debilitating diseases in more than 150 million people worldwide. The nematodes themselves play host to bacteria that live within their cells, but in this case, the relationship is classic mutualism, with each benefiting from the other. Indeed, the *Wolbachia* bacterium is so crucial to its host nematode that apparently eradicating it with antibiotics severely compromises the nematode's ability to complete its life cycle within its human host. Thus, understanding the details of this symbiosis may help identify new strategies for controlling diseases caused by filarial nematodes. In a new study, Barton Slatko and colleagues present the complete DNA sequence of the *Wolbachia pipientis* strain within *Brugia malayi*, a parasitic nematode responsible for lymphatic filariasis, and analyze its genome for clues to the interdependence of the two species.

This *Wolbachia* genome is small, only about a million base pairs, and many metabolically critical genes have degraded through mutation to the point of uselessness. This phenomenon, called reductive evolution, is typical of long-term symbioses, as the two partners increasingly complement one another's

biochemical activities, reducing the selection pressure on otherwise lethal mutations. *Wolbachia's* translational machinery and DNA repair equipment are largely intact. The bacterium appears to supply nucleotides to its host, as it contains complete pathways for biosynthesis of both purine and pyrimidine nucleotides. This is in contrast to *Rickettsia*, a close relative of *Wolbachia* and a mammalian parasite. Slatko and colleagues enumerate a variety of other pathways that have either been degraded or preserved, and highlight patterns in the genome structure through comparisons with both *Rickettsia* and another *Wolbachia* strain, found in fruit flies. For example, the two *Wolbachia* strains appear to have different membrane structures, possibly reflecting their different lifestyles (mutualistic versus parasitic).

*Wolbachia* can manufacture riboflavin and FAD, which are essential metabolic coenzymes and which do not appear to be made by its host. Conversely, it cannot synthesize amino acids and a variety of other vitamins and cofactors, and probably depends on the nematode to supply them. One discovery of possible significance is the presence in the bacterium of the synthetic pathway for heme—the oxygen-carrying iron component of hemoglobin. The nematode may require heme for synthesis of developmental hormones, so *Wolbachia's* heme pathway may be an inviting target for therapy against



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**Over a billion people are at risk for infection by filarial nematodes, parasites that cause elephantiasis (Photo: Dr. Steven A. Williams)**

nematode infection. Since no new antifilarial has been developed in two decades, these results may quickly lead to new therapeutic strategies against these parasites.

Foster J, Ganatra M, Kamal I, Ware J, Makarova K, et al. (2005) The *Wolbachia* genome of *Brugia malayi*: Endosymbiont evolution within a human pathogenic nematode. DOI: 10.1371/journal.pbio.0030121

## White Collar Proteins Help Fungi Do It in the Dark

DOI: 10.1371/journal.pbio.0030142

Fungi live mainly in the dark, and they like it that way—out of the sunlight, they can avoid desiccation and damage from ultraviolet rays. The ability to sense light, therefore, is adaptive for fungi of all kinds. A pair of light-sensing proteins had been identified in the model fungus *Neurospora crassa*, an ascomycete (one of the three fungal subgroups, defined by the production of sexual spores within sac-like structures), but little was known about mechanisms in other fungal phyla. In a new study, Alexander Idnurm and Joseph Heitman show that the basidiomycete (a subgroup defined by the production of sexual spores on the

ends of club-like structures) *Cryptococcus neoformans* employs a similar protein pair, which regulate mating, growth, and virulence of this human fungal pathogen.

In *N. crassa*, blue light is sensed by the protein White collar 1, which interacts with a flavin (light-absorbing pigment) tuned to photons in the blue region of the spectrum. White collar 1 then binds to White collar 2, and the complex serves as a transcription factor. In this study, the authors searched the *C. neoformans* genome for genes with similar evolutionary origins to these two genes (called homologs), as well as others implicated in light sensing, and identified

*Basidiomycete white collar 1*, or *BWC1*, along with other light-sensor candidates, including an opsin and a phytochrome homolog. Mutations of *BWC1*, but not the other candidate photoreceptors, rendered *C. neoformans* insensitive to light. While mating and fruiting in the wild-type fungus is suppressed by exposure to blue light, *bwc1* mutants were unaffected by light. Interestingly, the mating process was released from light inhibition when either one of the two mating strains were mutated, suggesting that the cell fusion process at the heart of fungal mating requires only one cell to commit to fusion. In addition, *bwc1* mutants were extremely

sensitive to ultraviolet radiation. No homolog of photolyase, a protein that uses light to repair DNA damage, was identified in the genome. Future studies will be necessary to understand how Bwc1 functions in ultraviolet resistance, but these findings suggest the protein could sense photons in both the ultraviolet and blue wavelengths.

To identify other proteins with which the Bwc1 protein functionally interacts, the authors examined nearly 3,000 mutant strains, yielding three with a phenotype similar to the *bwc1* mutant. They found that the gene for one of these, dubbed *BWC2*, is a homolog of *N. crassa* White collar 2, and that its protein binds to Bwc1. Together, the

two influence transcript levels of two key genes required for *C. neoformans* mating, further strengthening the case that the pair function as a transcription factor as do their homologs in *N. crassa*. Interestingly, mutants of either *BWC1* or *BWC2* were less virulent than the wild-type strain of the fungus, revealing a novel environmental signaling pathway involved in *C. neoformans* virulence.

The functional and structural similarities of the ascomycote and basidiomycote White collar proteins indicate that they arose prior to the split of these two lineages more than 500 million years ago. The fungal kingdom contains an estimated one million species. The authors suggest that the

ultraviolet protection afforded by the White collar system may have been crucial to the evolutionary diversification of this kingdom, in particular when ultraviolet radiation on the earth's surface was higher than it is today, such as when life emerged from the sea and colonized the barren continents. They also note that the same proteins are found in clinical isolates of *C. neoformans*, and that the mitigation of virulence by *bwc1* and *bwc2* mutations will be useful in the identification of new genes required for disease development in this important pathogen.

**Idnurm A, Heitman J (2005) Light controls growth and development via a conserved pathway in the fungal kingdom. DOI: 10.1371/journal.pbio.0030095**

## A New Role for a Protein Involved in Energy Metabolism

DOI: 10.1371/journal.pbio.0030133

Adjusting to life after birth takes a lot of energy. One way cells meet increased demand is by ramping up synthesis of mitochondria, the cells' power generators. This ability to increase mitochondria becomes limited in a variety of diseases including diabetes and heart failure. Therefore, it is important to identify the factors that control mitochondrial function. One way researchers have searched for candidate proteins that play a role in this process is by overexpressing proteins in targeted cells to see what happens. That's how several previous studies concluded that a protein called PGC-1 $\alpha$  triggers pathways that promote mitochondrial synthesis and regulate both mitochondrial activity and energy metabolism.

In a new study, Daniel Kelly and colleagues took a different approach. Rather than increasing the protein's activity, they blocked it. To do that, Kelly and colleagues engineered "knockout" mice that lack functional copies of the *PGC-1 $\alpha$*  gene. PGC-1 $\alpha$ , they found, isn't absolutely required for mitochondrial biogenesis but plays a vital role later in life by "boosting" the ability of cells to increase mitochondrial function in response to the shifting energy demands and physiological stresses encountered after birth.

Though leaner than the control mice soon after birth, by 18 weeks the female knockouts were slightly heavier and had more body fat, even though their food intake and activity levels matched the

controls. Knockout mice had observable growth defects in skeletal and heart muscle—tissues with high mitochondrial energy requirements—were less active and more easily fatigued than the controls, and had abnormal heart rates



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**PGC-1 $\alpha$  deficient mice can't keep pace (Photo: MedPic, Washington University)**

after physical exertion. And their livers showed a propensity to accumulate fat because of abnormal mitochondria.

Altogether, these results demonstrate PGC-1 $\alpha$ 's critical role in regulating the

adaptive metabolic responses required by the increasing energy demands and changing physiological stimuli associated with a growing organism. The increased fat stores and weight gain in the knockout mice, the authors

propose, could result from a systemic reduction in energy use, related to defective mitochondria. Given the recently reported link between *PGC-1 $\alpha$*  mutations and human obesity and diabetes, this connection will likely trigger further investigations. And given the pivotal role mitochondria play in a wide range of organs, this mouse model could help shed light on metabolic defects associated with a wide range of diseases.

Interestingly, another group, led by Bruce Spiegelman, reported on a *PGC-1 $\alpha$*  knockout model last year. Their mice share traits with the mice described here, but also exhibit a number of contrasting traits, including hyperactive, lean males, which the Spiegelman group attributed to a neurological defect. Kelly and colleagues speculate on possible causes for the differences in the results of the two studies, but only direct comparison of both mouse models will explain the inconsistencies.

**Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, et al. (2005) PGC-1 $\alpha$ -deficiency causes multi-system energy metabolic derangements: Muscle dysfunction, abnormal weight control and hepatic steatosis. DOI: 10.1371/journal.pbio.0030101**

## How a Latent Virus Eludes Immune Defenses

DOI: 10.1371/journal.pbio.0030149

For a virus to survive, it must elude the ever vigilant immune sentinels of its host. A latent virus can escape immune detection if it resides in nondividing cells and doesn't produce any proteins. No viral proteins means no red flags for immune cells. If the virus targets one of the many cell types that rarely divide, it's relatively safe while latent. But some viruses, like the gamma-herpesvirus, infect B cells of the immune system, which occasionally divide. The gamma-herpesvirus genome persists as circular pieces of DNA called episomes. When an infected B cell divides, the latent gamma-herpes virus episome must replicate and segregate into daughter cells along with the cell's genome. Viral replication and segregation requires the services of a protein called the episome maintenance protein—a potentially recognizable target for immune cells.

Gamma-herpesviruses, including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), can induce uncontrolled lymphocyte (immune cell) proliferation and result in lymphoma, Hodgkin's disease, and Kaposi's sarcoma. These diseases arise from the persistent latent infections that take hold after initial infections are controlled by immune defenses. The episome maintenance protein produced by EBV, called EBNA-1, harbors an amino acid element in its epitope—the region that binds to a T cell and triggers an immune response—that helps the viral protein evade the killer T cells that could destroy it. Lab studies show that the amino acid element limits EBNA-1's interaction with T cells by inhibiting synthesis and, to a lesser degree,

degradation of the protein. How this evasive action works or helps the virus in a living organism is not entirely clear. But if T cells aren't presented with bits of viral protein, they have no way of knowing the virus is present.

In a new study, Neil Bennett, Janet May, and Philip Stevenson explore this question by studying virus–host interactions in mice infected with the murine gamma-herpesvirus-68 (MHV-68). Though MHV-68 infects mice, it behaves similarly to EBV and KSHV infections in humans, producing an acute mononucleosis-like illness and a pervasive pool of latently infected B cells. The episome maintenance protein in MHV-68 and KSHV is called ORF73. None of the viruses can maintain latent infections with deficient episome maintenance proteins.

Stevenson and colleagues first demonstrated that ORF73 limits T cell recognition and then identified a key region responsible for immune evasion by modifying different regions of the viral protein. In the next round of experiments, the authors asked how the viral protein manages this feat. They discovered that ORF73 limits T cell recognition much like EBNA-1 does, by reducing synthesis and degradation of the protein. One region strongly associated with inhibiting epitope presentation to killer T cells corresponded to reduced protein synthesis. When the authors modified the ORF73 transcript to circumvent T cell evasion, the T cells “wiped out” latent virus. These results indicate that avoiding epitope presentation during episome maintenance is key to the virus's survival.

Interestingly, the MHV-68 episome



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### MHV-68 virions emerging from infected cells

maintenance protein mediates immune evasion even though it lacks the amino acid element that does the job for EBV. Future studies will have to determine the responsible MHV-68 epitope and the mechanisms that engineer immune avoidance. Since a majority of epitopes that killer T cells recognize come from aborted translation events, it may be that evasive action is taken at the RNA transcript stage, before RNA is translated into protein. Evading killer T cells, the authors argue, is key to the survival of the gamma-herpesvirus. By figuring out just how evasion occurs, scientists can identify a promising target for controlling infection.

Bennett NJ, May JS, Stevenson PG (2005) Gamma-herpesvirus latency requires T cell evasion during episome maintenance. DOI: 10.1371/journal.pbio.0030120

## Cracking the Olfactory Code

DOI: 10.1371/journal.pbio.0030122

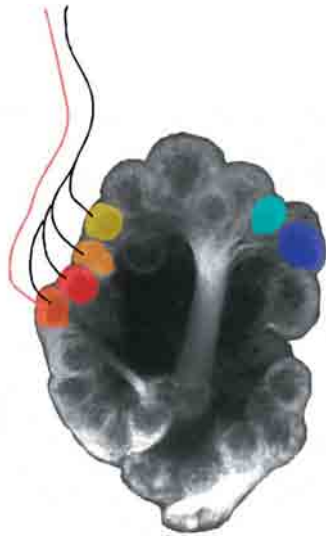
For Proust, a taste of cookie was enough to trigger vivid recollections of his childhood, the first of a long string of reveries that he fashioned into his famous memoir *Remembrance of Things Past*. For many animals, too, tastes and smells are evocative and play a crucial role in finding food, allowing them to build on past successes and to learn how to find their next meal.

To locate blooming flowers, for example, honeybees rely heavily on scent.

They can associate a whiff of an aldehyde, say, with a nectar-filled orchid. Then later they'll seek out the same or similar scents. To succeed in the wild, they must be able to distinguish relevant scents at varying concentrations, and within complex milieus of other scents. But to find food in varied conditions and adapt to new situations, they also have to generalize from past experience.

Through both physiological and behavioral studies, scientists have

investigated the response to smell in a wide range of organisms and have suggested that two key properties of scent-inducing chemicals are the functional class, such as alcohol or aldehyde, and the carbon-chain length. Bees trained to associate a particular chemical with a reward, for example, can then generalize to some extent to other chemicals with the same functional groups or similar carbon-chain lengths. In these situations, bees are surprisingly



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### Linking smell perception and neural activity in the bee (Image: Axel Brockmann)

consistent in both in their behavior (extending their proboscis to an odor previously associated with food) and in their brains (brain activity in smell-processing centers). Each set of data, behavioral and neural, can be thought of as a “code” underlying the bee’s response: present a scent, and a bee’s brain and

body will tend to react in a certain way.

A new study of smell perception in honeybees (*Apis mellifera*) published in *PLoS Biology* gives a more comprehensive picture of how bees react to a suite of scents and also shows a remarkable correspondence between the codes for the insects’ behavior and brain activity. The researchers, led by Martin Giurfa, first trained bees to associate a specific chemical, such as the alcohol 1-nonanol, with a sucrose reward. Then the researchers tested the bees’ response to a set of other chemicals, varying in carbon-chain length from six to nine, and with four different functional groups: aldehydes, ketones, and primary and secondary alcohols.

By watching how often the bees generalized—that is, how often they responded positively to a particular scent when they’d been trained on another—the researchers could assign perceptual “distances” between pairs of chemicals. Drawing together all these distances, they created a preliminary map of the bees’ “perceptual space,” similar to how surveyors measure distances between landmarks to map a landscape. From this comparison they found, for example, that the bees generalized more by functional

group than by carbon-chain length.

Previously, Giovanni Galizia’s group, which works closely with Giurfa’s group, had recorded bees’ brain responses to the same pairs of scents, assigning distances within centers of activity for each scent. Giurfa’s team compared these two sets of data and found that the perceptual and neural distances correlated well, which suggests there’s a species-specific code that ties together the insects’ brain and behavior.

The brain recordings covered only a quarter of the bees’ main smell-processing center, the antennal lobe. Future studies with new methods of microscopy that visualize more of the brain and which focus on the olfactory message sent by the antennal lobe to higher-order brain centers should only improve our ability to investigate the correlations between brain and behavior, the authors say. Such studies would go even further toward cracking the codes underlying animals’ perception and memory.

**Guerrieri F, Schubert M, Sandoz J-C, Giurfa M (2005) Perceptual and neural olfactory similarity in honeybees. DOI: 10.1371/journal.pbio.0030060**

## A Small RNA That Neutralizes a Protein Linked to Tumor Development

DOI: 10.1371/journal.pbio.0030147

For most of human history, cancer has been incurable. But with the invention of anesthesia in the mid-19th century, surgeons were able to remove some forms of cancer surgically. Radiotherapy arrived next, soon after the discovery of X rays in 1896. Chemotherapy, now a mainstay of cancer treatment, did not arrive until the mid-1940s, when nitrogen mustard, an alkylating agent related to the mustard gas used in the two World Wars, was developed as an anticancer agent. Unfortunately, although cancer cells are hypersensitive to the effects of alkylating agents—molecules that introduce lethal changes into the cell’s DNA—normal cells are also targeted by them, although less damage is caused because normal cells typically divide slower than cancer cells. Using chemotherapy based on alkylating agents to treat cancer is like using a sledgehammer to crack a nut. But with improved knowledge about how cancer cells differ from normal cells, chemotherapeutics are now being

designed that hit only cancer cells.

Many of these new chemotherapeutics target protein receptors called tyrosine kinases. These receptors, which sit on the cell surface, normally stimulate intracellular pathways that control proliferation and other cellular functions in response to growth factors. In tumors, these receptors often have mutations that allow them to become active without growth factor binding, which results in the uncontrolled proliferation that is characteristic of cancer cells. For instance, mutations in the RET receptor tyrosine kinase are responsible for multiple endocrine neoplasia (MEN) type 2 syndromes. Whereas external stimulation by a growth factor is normally needed before two RET molecules can bind together (a process called dimerization) to activate intracellular signaling cascades, in MEN type 2A, a mutation in the RET receptor tyrosine kinase provokes (or induces) dimerization without external stimulation.

In recent years, several proteins and

various small synthetic chemicals have been designed that specifically inhibit the activity of mutated receptor tyrosine kinases and show anticancer activity. Domenico Libri and colleagues are now working on another class of molecules, called aptamers, that have potential as anticancer drugs. Aptamers—single-stranded nucleic acid molecules that are 50–100 bases long and can be selected for their ability to bind directly and tightly to specific proteins—are less likely to be targeted and destroyed by the body’s natural defenses than some other types of potential therapeutic molecules.

To find an aptamer able to recognize the RET receptor kinase within a cellular membrane environment, the researchers used whole-cell SELEX (systematic evolution of ligands by exponential enrichment), a process in which large pools of oligonucleotides are enriched for molecules that can distinguish between a real and sham target. First, they incubated a large pool of RNAs with PC12 cells, a rat cell line not expressing

RET, to remove sequences binding non-specifically to the PC12 cell surface. Unbound sequences were recovered and applied to PC12 cells expressing human RET with the MEN type 2A mutation that causes dimerization. This time, bound sequences were retained, and the whole selection process was repeated another 14 times to select for aptamers that recognize the dimeric form of the RET extracellular domain.

Of the 67 sequences pulled out of the final pool of RNAs, the researchers found one sequence, D4, that not only bound the extracellular domain of RET but also blocked RET downstream signaling events and subsequent cellular and molecular changes. The researchers



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#### A newly synthesized molecule, D4, inhibits cellular differentiation

suggest that D4 blocks the dimerization-dependent activation of RET—whether it's induced by its physiological signaling molecule or by an activating

mutation—and suggest that their method can be used to identify macromolecules with potential therapeutic effects against other transmembrane receptors involved in tumorigenesis, particularly since the whole-cell SELEX approach should efficiently select aptamers that recognize these receptors as they are found on the surface of tumor cells.

Cerchia L, Ducongé F, Pestourie C, Boulay J, Aissoumi Y, et al. (2005) Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. DOI: 10.1371/journal.pbio.0030123

## The Bacteria's Guide to Survival

DOI: 10.1371/journal.pbio.0030140

From *The Worst Case Scenario Survival Handbook*—with handy entries like “How to escape from killer bees” and “How to escape from quicksand”—to *The Zombie Survival Guide: Complete Protection from the Living Dead*, survival guides are one of the latest publishing fads.

If there was a market for it, a survival guide for bacteria might include topics like “How to use your pili to keep your host from going apoptotic.” A host's cells can respond to a bacterial infection with apoptosis, or programmed cell death. For bacteria that pass directly from host to host, this can pose a problem. If the bacteria are highly virulent and induce too much cell death, they could take down their host before they're able to jump ship, thus hurting the bacteria's chances of survival in the long run.

Earlier studies suggested that bacteria can use their pili, finger-like appendages that many bear on their surface, to pull on a host's cell membranes and thus influence the cell's behavior. But these studies, which looked at mutant bacteria that could not retract their pili, did not examine the matter of how the bacteria coax their hosts to stay alive.

Now, in *PLoS Biology*, a group of researchers present more direct evidence that bacteria can induce changes in

hosts' gene expression—and possibly keep the host cells alive longer—through tiny tugs on cell membranes. The study, led by Magdalene So, examined gene activity in human epithelial cells infected with *Neisseria gonorrhoeae*, the bacteria responsible for the sexually transmitted disease gonorrhea.

By comparing cells infected with normal *N. gonorrhoeae* to those infected with a mutant strain with defective pili, the researchers found a subset of 52 host genes that had higher activity when the host was infected with the normal bacteria, suggesting that the pulls of the pili were responsible. They also ran a key control experiment with an artificial mechanical pull on the host cell membrane. By coating magnetic beads with a preparation of bacterial pili, the beads attached themselves to the cell membranes. Then, in the presence of a magnetic field, the beads tugged on the cell membrane, approximating the effects on gene expression during infection with normal bacteria.

Thus, the mechanical tugs seem responsible for triggering a signaling cascade in the host cells, which ultimately affects the host's gene expression. Many of the genes that increased in activity due to the tugs were already known to

regulate apoptosis and cellular response to stress, including mechanical strain on the membrane. Also, a majority of these genes were known to be induced by a family of proteins called mitogen-activated protein kinases, or MAPKs. The researchers showed that blocking MAPKs reduced the activity of several of the genes that are usually enhanced by infection with the normal bacteria. Also, they found that cells infected with the bacteria tended to survive treatment with staurosporine, a chemical that normally induces apoptosis.

Overall, the group's findings support previous speculations that some bacteria influence gene expression and the fate of cells in their hosts by tugging on the host cells' membranes with their pili. For bacteria like *N. gonorrhoeae* that pass directly from host to host, the researchers argue, it would be in a bacterium's interest to help keep its host alive. And bacteria appear to do this with the help of their pili.

Howie HL, Glogauer M, So M (2005) The *N. gonorrhoeae* type IV pilus stimulates mechanosensitive pathways and cytoprotection through a *pilT*-dependent mechanism. DOI: 10.1371/journal.pbio.0030100



## Heart Repair Gets New Muscle

DOI: 10.1371/journal.pbio.0030125

When you think of the cell as the fundamental unit of life, it's not surprising that some organs deal with injury better than others. A flesh wound or muscle tear might hurt, but, assuming you are otherwise healthy, both will heal. The prognosis for a heart attack, on the other hand, is not so clear-cut. What accounts for the difference?

Skin cells reproduce regularly to replace dead cells, and simply increase production in the event of injury. Skeletal muscles recruit new muscle cells from a type of precursor cell within the muscle, called satellite cells, to repair a tear. Cardiac cells (cardiomyocytes), it has long been thought, appear to lack this capacity for self-renewal and repair, impeding the chances of a full recovery. That's why therapies derived from stem cells—which retain a unique ability to morph into any of the body's 200-plus cell types—hold such promise. But stem cells are a hot-button issue in the United States, complicating efforts to explore this promise.

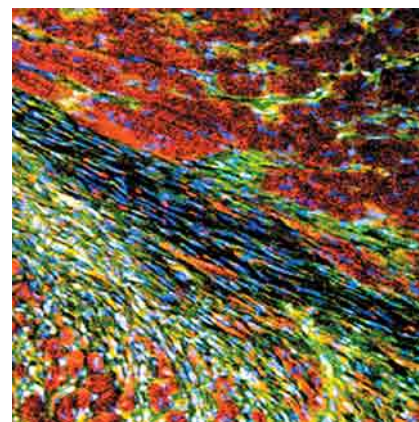
Recent evidence suggests that the heart might harbor stem cells after all and that such cells can be transformed into cardiomyocytes. In a new study, Neal Epstein and colleagues report that cells isolated from the skeletal muscle of adult mice can turn into beating cardiomyocytes in a test tube within days of isolation—and without the addition of gene-altering drugs or special cardiac factors. When freshly isolated cells (called skeletal precursors of cardiomyocytes, or Spoc cells) are injected into the tail veins of mice with heart damage, they migrate to the damaged tissue and differentiate into cardiac muscle cells.

What distinguishes a cardiomyocyte from a skeletal muscle cell? Specialized cells produce unique proteins, allowing scientists to use those proteins as

identifying markers. The so-called Spoc cells do not express any of the usual markers associated with either skeletal muscle satellite cells or partially differentiated skeletal muscle cells. By day 7 in culture, Spoc cells have undergone several rounds of cell division and have begun to express a (mostly) cardiac-specific protein, and have formed clusters of cardiac precursor cells, some of which beat. These precursors in turn express other cardiac-specific proteins.

Epstein and colleagues further divided Spoc-derived precursor cells into two groups based on whether or not they expressed another protein marker (Sca-1, a common marker found on blood stem cells). About 80% of cells without this protein differentiated into immature beating cells after proliferating for seven to ten days. They remained in an immature state (round and loosely attached) for over two months in culture, but differentiated into mature beating heart cells (elongated and adherent) when mixed with Sca-1 cells. The authors use video microscopy to track the cells' progression to beating cells, complete with contraction-generating thick myosin filaments that are "nearly identical" to those seen in developing cardiomyocytes. Epstein and colleagues also demonstrate that the Spoc cells are distinct from stem cells cultured out of bone marrow, heart, or fat tissue—sources of beating cells in other studies. The authors also injected these Spoc cells into mice with acute heart lesions to test the cells' ability to integrate into the damaged tissue. Many cells successfully migrated to and engrafted into the site of injury; some of these cells developed into cardiomyocytes. The cells showed a similar, though less robust, response to an older heart injury.

Epstein and colleagues argue that Spoc



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### Spoc cells can help repair a damaged heart

cells are more likely to be precursors to cardiomyocytes than to be some other type of skeletal muscle stem cell. This is based on an absence of protein markers for skeletal muscle or skeletal satellite cells in Spoc cells, as well as the fact that Spoc-derived cells display spontaneous rhythmic beating and express cardiac markers, whether they are grown in a test tube or have migrated to injured hearts in study mice. The authors can't say why skeletal muscle would harbor cardiac stem cells or why so few of these cells pitch in to repair a cardiac injury. But for now, the Spoc cells provide a valuable tool for studying heart cell differentiation. And with time, they might prove an important resource for developing cell-based therapies for heart disease. See also the Primer "Alchemy and the New Age of Cardiac Muscle Cell Biology" (DOI: 10.1371/journal.pbio.0030131).

Winitsky SO, Gopal TV, Hassanzadeh S, Takahashi H, Gryder D, et al. (2005) Adult murine skeletal muscle contains cells that can differentiate into beating cardiomyocytes in vitro. DOI: 10.1371/journal.pbio.0030087

## Memories Are Made of This: Modeling the CaMKII Molecular Switch

DOI: 10.1371/journal.pbio.0030124

We all know that our memories are stored somehow in our brains. But exactly how do we remember the way to our office or what our mother looks like or the date we got married? Scientists attribute our ability to store apparently infinite numbers of memories for decades to long-lasting changes in the electrical,

structural, and biochemical properties of neurons. One cellular mechanism proposed to be involved in the storage of memories—long-term potentiation—involves alterations in the strength of messages passed from one neuron to another across structures known as synapses.

The initiation of long-term potentiation is caused by activation of N-methyl-D-aspartate receptors on the receiving neuron and a subsequent increase in the intracellular calcium concentration in a region of the neuron that is called the postsynaptic density. The increase in calcium, in turn, activates the calcium/

calmodulin-dependent protein kinase II (CaMKII). This enzyme seems to play a critical role in long-term potentiation, and has been proposed as one of the leading candidates to act as the molecular switch that maintains stable synapse-specific cellular changes. To fulfill this role, CaMKII would need to have stable UP and DOWN positions, or states, much like a light switch.

Xiao-Jing Wang and colleagues now provide a new analysis that strengthens the argument that CaMKII is a molecular switch involved in the storage of long-term neural changes. The activity of the CaMKII holoenzyme (the complete enzyme consisting of both regulatory and catalytic subunits) is controlled by its autophosphorylation state—the enzyme is able to add phosphate groups to specific amino acids within itself. Previous modeling studies have shown that the interplay between the autocatalytic addition of phosphate groups to CaMKII and the removal of phosphate groups by protein phosphatase-1 (PP1) enzymes produces two stable states of the CaMKII enzyme at basal free calcium levels. The DOWN state is unphosphorylated; the

UP state is highly phosphorylated. When there is a transient high input of calcium, as happens when long-term potentiation is induced, the CaMKII enzyme flips from a DOWN state to a persistent UP state.

The questions that Wang and colleagues have now asked are what factors affect the stability of the state of this switch, and how many CaMKII holoenzymes are needed to construct a switch that could last a lifetime. These questions are important because a switch that could be spontaneously reset by small, random fluctuations of the conditions within the postsynaptic density would not be useful in maintaining stable long-term changes. The researchers have used a mathematical probabilistic modeling technique known as Monte Carlo simulation, together with the known biochemical and thermodynamic characteristics of CaMKII and PP1, to test how random fluctuations in the chemical reactions involved in the CaMKII/PP1 system change the state of the switch.

They report that switch state stability requires a balance between the phosphorylation and dephosphorylation

rates of CaMKII, and that the turnover rate of the kinase—the replacement of old molecules with new ones—critically affects switch stability. However, their main finding is that the lifetime of states of the switch increases exponentially with the number of CaMKII holoenzymes that are present. This finding is important because experimental work by other researchers has estimated that there are about 30 CaMKII holoenzymes present in a typical postsynaptic density, and until Wang's team did their modeling it was unclear whether this number of holoenzymes could build a switch stable enough to last a lifetime. In fact, Wang and co-workers estimate that a switch containing as few as 15 holoenzymes can remain activated for longer than a human lifetime. Thus, the researchers conclude, CaMKII switches may indeed play a critical role in preserving our precious memories throughout our lives.

**Miller P, Zhabotinsky AM, Lisman JE, Wang XJ (2005) The stability of a stochastic CaMKII switch: Dependence on the number of enzyme molecules and protein turnover. DOI: 10.1371/journal.pbio.0030107**

## Killers on Patrol: Liver Lymphocytes Remain in the Blood Vessels

DOI: 10.1371/journal.pbio.0030154

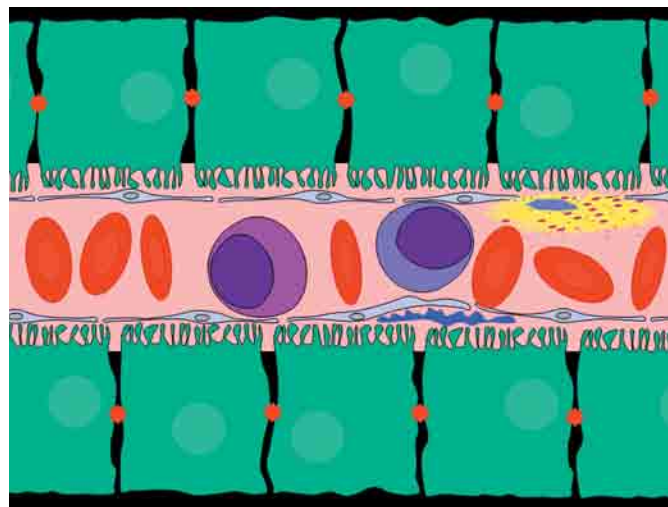
Protecting the liver from infection presents the immune system with a tough challenge. Because it is the first stop for food absorbed from the gut, the liver is constantly bathed in a rich broth of mostly harmless foreign molecules, which need to be immunologically tolerated. On the other hand, the slow rate of blood flow and high internal surface area makes the liver's many sinuses ideal sites for infections to take hold. One unusual type of immune cell patrolling this complex maze in the liver is the natural killer T (NKT) cell. In this issue, Dan Littman, Mike Dustin, and colleagues shed some light on the behavior of this little-appreciated and important hepatic guardian, showing its unexpected ability to perform immune surveillance entirely within the vasculature. NKT cells crawl along vascular passages in the liver (hepatic sinusoids) at high speeds for

a cell moving under its own power, but slow speeds compared to blood flow, and never or rarely venture into the surrounding tissue.

To track the movements of these cells, the authors used a mouse in which the gene for the CXCR6 receptor was replaced with a gene for green

fluorescent protein (GFP). In these mice, cells that express CXCR6 also express GFP, and because they fluoresce, they can be visualized and tracked under a microscope. The study showed that cells remained entirely within the blood vessels, crawling along the endothelial lining at approximately 16 microns per

minute. Despite directional blood flow, the NKT cells moved and changed directions randomly, and could occasionally be seen migrating past one another in opposite directions within a single vessel. When the authors injected antigen into the blood stream, the cells abruptly stopped, and remained stationary, suggesting that they had fulfilled their first duty to find antigen and were beginning their next: to alert the rest of the immune system. Surprisingly, antigen detection occurred within the blood stream—it is normally



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**Tracking the movement of glowing T cells in the vasculature**

thought to occur after lymphocytes leave the blood.

While they make up less than 1% of lymphocytes in other tissues, NKT cells comprise 30% of the lymphocytes in the liver. Mice lacking both copies of the *cxcr6* gene had only one third the normal number of hepatic NKT cells. Though chemokine receptors are typically thought to function by directing cell homing, the authors observed that CXCR6-deficient cells died rapidly in vitro, suggesting that CXCR6 delivers survival signals and thereby controls the number

of hepatic NKT cells and liver immune surveillance in general.

While cell numbers were reduced, the movement of the remaining cells in the homozygous-deleted mice was no different than that in the heterozygotes. Because of this, the authors propose that cell motility in wild-type mice is likely to be similar to what they observed in these experiments. Based on the speed and density of the NKT cells, they calculate that each cell can visit a new hepatocyte every two minutes, and that every hepatocyte is surveyed approximately

once every 15 minutes. This is in stark contrast to surveillance in the lymph nodes, in which a typical dendritic cell receives 5,000 visits per hour from its resident T cells, but it may make biological sense given that NKT cells have a much more restricted range than conventional T cells.

**Geissmann F, Cameron TO, Sidobre S, Manlongat N, Kronenberg M, et al. (2005) Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. DOI: 10.1371/journal.pbio.0030113**