The Cyanophage Molecular Mixing Bowl of Photosynthesis Genes

Emma Hill  |  DOI: 10.1371/journal.pbio.0040264

Among the wealth of microbial organisms inhabiting marine environments, cyanobacteria (blue-green algae) are the most abundant photosynthetic cells. Prochlorococcus and Synechococcus, the two most common cyanobacteria, account for 30% of global carbon fixation (through the photosynthetic process in which sugars are manufactured from carbon dioxide and water). By drawing on natural resources, these microbes use photosystems (PS) I and II (the two reaction centers in photosynthesis) to harness energy.

Intriguingly, some viruses that infect cyanobacteria (called cyanophage), carry genes that encode two PSII core reaction-center proteins: PsbA (the most rapidly turned over core protein in all oxygen-yielding photosynthetic organisms) and PsbD (which forms a complex with PsbA). By expressing their own copies of psbA and psbD during infection, these cyanophages have managed to co-opt host genes to suit their own purposes: enhancing photosynthesis. It seems likely that they do this in the interests of their own fitness, since cyanophage production is optimal when photosynthesis is maintained during infection.

Until recently, only a small sample of cyanophages had been examined, leaving open the questions of how widespread PSII genes are in these organisms and where the genes came from. To answer these questions, Matthew Sullivan, Debbie Lindell, Sallie Chisholm, and colleagues examined a pool of 33 cyanophage isolates (cultured from samples collected from the Sargasso Sea and the Red Sea), along with data already available for nine other cyanophages, for the presence of psbA and psbD genes. They found psbA was present in 88% and psbD in 50% of the cyanophages studied. By analyzing the sequences of these genes along with those from Prochlorococcus and Synechococcus host genes, they reconstructed the evolutionary history of how the PSII genes entered the phage genomes.

Cyanophages are divided morphologically into three main families (Podoviridae, Myoviridae, and Siphoviridae). Looking at the distributions of the PSII genes across the different families, Sullivan, Lindell, et al. saw that psbA was present in all myoviruses and all Prochlorococcus podoviruses, but not in Prochlorococcus siphoviruses or Synechococcus podoviruses. The high levels of sequence conservation between the different cyanophages suggest that this gene is probably functional and that it is likely to increase the reproductive fitness of the phage. The length of the latent period may impact the distribution pattern of psbA among these phage groups. However, more information about the physiological characteristics of cyanophages is needed to further investigate these possibilities.

The second gene, psbD, was less prolific but was seen in four of the 20 Prochlorococcus myoviruses and 17 of the 20 Synechococcus myoviruses examined—all of which also encoded psbA. Myoviruses are known to infect a wider range of cyanobacteria than the other cyanophage families. Indeed, when investigated, the psbD-encoding myoviruses correlated with those known to have a broader host range. Perhaps the co-opting of both PSII genes ensures a functional PsbA–PsbD protein complex to enhance infection for these cyanophages that are able to infect a wider range of hosts.

To determine when the PSII genes had been transferred into the phage and from where, Sullivan, Lindell, et al. investigated the nucleotide sequences of psbA and psbD from both Prochlorococcus and Synechococcus host and cyanophage. Using meticulous sequence analyses and standard statistical methods, they generated phylogenetic trees to explain the evolutionary history of these two PSII genes.

By analyzing the clusters of sequence types within the resulting tree, the authors saw evidence that psbA was transferred from the cyanobacteria host genome into the phage genome on four independent occasions and two separate occasions for psbD. Exchange events were generally host-range specific, meaning that Prochlorococcus genes transferred to Prochlorococcus phages, and so on. However, a few intriguing exceptions, where genes did not cluster with their hosts, were observed; these might result from genetic exchange between members of different phage families (one of broader host range) during co-infection of the same host.

Sullivan, Lindell, et al. were also able to use their dataset to investigate a previous suggestion that alterations in the nucleotide distributions within individual PSII genes (creating a kind of patchwork gene) demonstrate that intragenic recombination has taken place. Indeed, they confirm that this occurs among Synechococcus myoviruses.
and Prochlorococcus podoviruses. In some cases involving Synechococcus, intragenic recombination appears to have happened in both host-to-phage and phage-to-host directions for both genes; and, for some Prochlorococcus genes, DNA from an unknown source also seems to have been inserted. Occasionally, intragenic exchanges are also seen between Synechococcus hosts.

The authors compare their cultured results to those from wild phage sequences from the Pacific Ocean and see that much of the natural diversity is similar to the sequences from the cyanophage isolates, despite their origination from different ocean basins. Overall, therefore, a considerable amount of genetic shuffling takes place within these two PSII genes in cyanophages, and this creates a reservoir of photosynthetic diversity from which both host and phage are likely to benefit. This study offers a compelling example of global-scale microbial and phage co-evolution that likely influences the biological success of these prolific marine organisms.

Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, et al. (2006) Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. DOI: 10.1371/journal.pbio.0040234

Smashing Protein Complex Ions to Bits Reveals Their Structural Organization

Liza Gross | DOI: 10.1371/journal.pbio.0040287

Most cell diagrams ably illustrate the cell’s major structural elements, but they can’t convey the incredibly dynamic nature of cellular life. One cell can contain millions of ribosomes, each churning out a protein a minute. To make way for new proteins, obsolete proteins must be removed. Short-lived and misshapen proteins get tagged with ubiquitin proteins and sent to the proteasome, a massive enzyme complex that degrades proteins into peptides through a process called proteolysis.

First isolated from yeast, the “26S” proteasome (named after a measure of its macromolecular size) consists of two major subcomplexes, the 20S proteolytic core and the 19S regulatory particle. The 19S particle forms a cap over the 20S core; it has a base that binds to the core and a lid that sits atop the base. After the 19S particle recognizes ubiquitinated substrates and removes their ubiquitin tag, they’re unfolded and sent to the core for degradation. To better understand how this important regulatory complex functions, researchers need precise information on its structure—no small task, given that the lid’s many subunits presumably undergo dynamic assembly in the cell.

In a new study, Michal Sharon, Carol Robinson, and colleagues present an innovative approach for identifying the structural composition and organization of intact macromolecular complexes. By combining this approach with chemical cross-linking, which provides additional artificial bonds between the 19S components, the researchers were able to determine the lid’s structural organization and gain insight into the likely functional interactions between the components.

Sharon et al. first isolated the intact 19S complexes from yeast, then “electrosprayed” the macromolecules, converting them into ions for mass spectral analysis. Mass spectrometry reveals the protein composition of a sample based on its molecular mass. (Ions travel through a “time-of-flight” analyzer, which times the speed of ions as they travel through an electric field; the sorted ions reach a detector that shows the relative abundance of different masses in the sample as peaks across a spectrum.) The resulting mass spectrum confirmed previous reports that the lid consists of nine protein components, and indicated the presence of an intact complex (roughly 376 kiloDaltons) as well as a smaller subcomplex (roughly 185 kiloDaltons).

To further characterize these components, Sharon et al. used tandem mass spectrometry (MS/MS), a technique that involves multiple steps of sorting ions to isolate and analyze the complex’s components. The ionized sample accelerates through a gas-filled “collision chamber.” As multiple collisions yield increasing energy, the ionized sample (precursor ion) dissociates into “product ions” (individual subunits). Using this approach on the intact lid, the researchers could infer how the individual pieces of the lid fit together by identifying which of the components stuck together longer. From one round of MS/MS, they identified the two proteins Rpn9 and Rpn12. Then a second, higher energy round of MS/MS triggered the dissociation of a...
third protein, Rpn6. The researchers concluded that Rpn6, Rpn9, and Rpn12 interact weakly around the lid’s periphery. Yet another round of MS/MS, this time on the lid subcomplex, identified Rpn5, Rpn6, and Rpn9 as subunits that can form different interactions with Rpn8, a subunit that had not been detected by itself. Finally, using a technique called chemical cross-linking (which creates covalent bonds between neighboring proteins so they can be examined), the researchers identified additional interaction partners within the lid.

Based on the results of their MS/MS and cross-linking experiments, along with previously published data, the researchers proposed a model of how all these components come together to form the lid complex. The lid consists of two subcomplexes—one comprising Rpn5, Rpn6, Rpn8, and Rpn9; the other of Rpn3, Rpn7, Rpn12, and Sem1—with a link forged between Rpn5 and Rpn3. Rpn11, the enzyme responsible for coupling deubiquitination with degradation, is likely recruited by the first subcomplex. These insights into the configuration and possible role of the lid’s subunits will facilitate future investigations into the function and regulation of the 19S lid. And this technique—which can identify the subunit organization of intact complexes based on their mass spectra—should illuminate the architecture of a diverse range of macromolecules still uncharacterized.

Sharon M, Taverner T, Ambroggio XI, Deshaies RJ, Robinson CV (2006) Structural organization of the 19S proteasome lid: Insights from MS of intact complexes. DOI: 10.1371/journal.pbio.0040267

Accelerated Growth following Poor Early Nutrition Impairs Later Learning
Liza Gross | DOI: 10.1371/journal.pbio.0040270

The conditions an organism experiences early in life can have critical impacts on its subsequent health and well being, both over the short and long term. Aside from facing a greater risk of death, low birth weight human babies (5.5 pounds and under) have increased risk of developmental disabilities throughout life. Recent evidence indicates that many organisms can offset some of the changes associated with early poor nutrition by modifying their physical development. For example, poorly nourished children can undergo a period of accelerated growth once their diet improves, ultimately appearing normal as an adult.

But such compensatory measures often come at a price, with cognitive or other developmental disabilities emerging later in life, suggesting that growth rates are optimized to avoid such costs. Poor nutrition early in life can impair neural development, leading to lower IQ in humans and flawed song learning in birds. A recent study found that full-term, low birth weight babies who grew quickly when fed an enriched diet had lower cognitive skills when tested at nine months than did babies given a normal diet. But questions remain about the relative consequences of compensatory growth versus impaired growth and poor nutrition on the observed cognitive defects.

In a new study, Michael Fisher, Rudolph Nager, and Pat Monaghan explored the connection between early poor nutrition, compensatory growth, and learning ability in adulthood. To circumvent the confounding variables inherent in human studies and to control for genetic effects, the researchers compared the learning performance of zebra finch siblings reared on different quality diets after hatching. Only food quality, not quantity, was changed. The rate at which adult birds could learn a simple task, they found, depended on the rate of compensatory growth the birds showed following a period on lower-quality food early in life—not on the diet itself or on the degree of stunted growth.

Because zebra finch hatchlings are totally dependent on their parents for food, the researchers could vary food quality for the chicks by manipulating the food available. The birds, facile learners that are often used in cognitive studies, were reared on ad libitum diets of different nutritional quality and then tested for learning performance as adults. (Handily, zebra finches show little sex differences in size as adults.)

After hatching, siblings were raised on either a normal or low-quality diet for 20 days, then switched to the higher-quality standard diet. While on the low-quality diet, birds grew slower and were lighter than their control siblings by the end of the 20 days. Once they were switched to the standard diet, birds reared on the poor diet then grew significantly more than their normally fed siblings and reached the same adult size.

The extent to which birds’ growth was depressed during the poor nutrition phase of the experiment varied considerably, as did the degree of accelerated growth after the switch to a normal diet. As it happened, birds with the most stunted growth (relative to their control siblings) and those with the most accelerated growth (after switching diets) fell into different groups, allowing the researchers to distinguish cognitive effects associated with stunted growth from those associated with compensatory growth.

To test the adult birds’ learning performance, the researchers tested them on an associative learning task. Birds were placed in a circular foraging area with corridors leading to a screen with cups of seed behind it, and were trained to associate a yellow screen with food. Though all the birds eventually learned the task, their learning rate depended on the rate of compensatory growth they had undergone as.
Cultivating Bacteria’s Taste for Toxic Waste

Liza Gross | DOI: 10.1371/journal.pbio.0040282

More than 50 years after developing the nuclear bomb, the US Department of Energy is still grappling with the toxic consequences of nuclear weapons production. By the agency’s own reckoning, more than 2,500 billion liters of groundwater and 200 million cubic meters in 30 states and territories are contaminated with uranium. Based on increasing evidence that microorganisms can transform contaminants as a natural by-product of cellular metabolism, the agency launched an ambitious program in 1995 aimed at harnessing natural biogeochemical processes to clean up radioactive sites.

Dissimilatory metal-reducing bacteria (DMRB) can convert soluble radioactive uranium into an insoluble, or solid, form called uraninite. The soluble form moves through groundwater with relative ease; the insoluble form can stick to soil particles and is far less mobile, significantly reducing the probability that uranium will reach surface water or aquifers used for domestic water supply. But, before the bioremediation potential of DMRBs can be exploited, scientists must work out many details of uranium reduction by these microbes and determine the best ways to increase their fancy for toxic heavy metals. In a new study, Matthew Marshall, James Fredrickson, and colleagues examine the mechanisms of uranium reduction in a microbe that’s widely used in environmental research called Shewanella oneidensis strain MR-1. Using diverse genetic and microscopy techniques, they show that two polyheme ε-type cytochromes, MtrC and OmcA—proteins known to be involved in iron and manganese reduction—are also required for the reduction of uranium to insoluble uraninite.

S. oneidensis has a remarkably flexible respiratory network. When oxygen is available, the microbe obtains energy by oxidizing organic compounds to carbon dioxide, using oxygen as the electron receptor. When oxygen is scarce—in heavy-metal-laden sediments, for example—the microbe oxidizes organic compounds to carbon dioxide by using metals such as iron or uranium as electron acceptors. These “redox” reactions transfer electrons from one molecule (which is oxidized) to another (the reduced “terminal electron acceptor”) with the help of cytochromes as part of the terminal reductase complex.

Because uraninite accumulates on the surface of the microbe’s outer membrane, Marshall et al. reasoned that outer membrane cytochromes (OMCs) might contribute to the formation of uraninite nanoparticles. To investigate the role of OMCs in uranium reduction, they first studied a S. oneidensis mutant lacking the ability to produce functional ε-type cytochromes (the microbe has 42 genes predicted to make ε-cytochromes). This mutant failed to reduce uranium during a 48-hour period, whereas the nonmutant microbe reduced equivalent amounts of uranium totally in 24 hours. The researchers also created four OMC deletion mutants; those lacking either MtrC or OmcA, or both OMCs, had significantly slower uranium reduction rates. The fact that MtrC and OmcA affect uranium reduction rates suggests both proteins are important, even though in vitro experiments showed that only MtrC can function as a terminal reductase to transfer electrons to uranium. Deleting another OMC, mtrF, had little effect on reduction rates, which was not surprising, since this gene has never been linked to electron transfer to metals.

The researchers next used microscopic analysis on nonmutant and mutant microbes to determine what effect the OMC deletions had on the cellular location of uraninite particles. In nonmutants, uraninite is found localized both extracellularly and between the cell’s inner and outer membranes (the periplasm). A large proportion of the extracellular uraninite was densely packed in association with a complex called extracellular polymeric substance (EPS). In contrast, OMC mutants typically accumulated more uraninite in their periplasm rather than consorting with EPS outside the cell. Mutants lacking just MtrC or both MtrC and OmcA also revealed the most significant differences in the abundance, distribution, and density of uraninite–EPS complexes.

X-ray fluorescence microscopy revealed that elemental iron was closely...
Melanopsin Photopigment Comes in Two Distinct Forms

Mason Inman  DOI: 10.1371/journal.pbio.0040263

Photoreceptors aren’t just for seeing. Deep within the retina of the vertebrate eye are photoreceptors that capture light to regulate non-visual processes such as day-night rhythms and the narrowing and widening of the pupil. To capture light, these special cells—called intrinsically photoreceptive retinal ganglion cells—require pigments called melanopsins, similar to the opsins that the rod and cone cells use for turning light into vision. While the rod and cone opsin proteins have been extensively studied, much less is known about the melanopsins, although they appear to function more like the opsins found in invertebrate eyes.

Now a new study shows that there are actually two distinct versions of melanopsin. The two genes and their associated proteins have persisted through hundreds of millions of years of evolution—only for one to be lost in the lineage leading to mammals. The discovery, by James Bellingham, Robert Lucas, and colleagues, clears up one set of questions, about the conservation of melanopsin genes through evolution. But it also opens up new questions about how the two related melanopsin genes and their associated proteins have persisted through evolution—only for one to be lost in the lineage leading to mammals. For both

The first known melanopsin gene was discovered in the African clawed frog, *Xenopus laevis*. Comparing the sequence of the frog version of the gene with that of humans and mice, it appeared that the gene had been poorly conserved through evolution. The central core regions (the area most critical for function) of the *Xenopus* and human melanopsin proteins are only 55% identical, whereas the corresponding portions of their rod opsin sequences are 85% identical. This posed a conundrum: even though their sequences differed, the melanopsins were somehow related to each other, and seemed to have an important function in these diverse organisms.

Bellingham and colleagues found that the *Xenopus* and human melanopsins had been distinct for eons. The researchers trawled databases, and identified and sequenced new melanopsin genes from a wide variety of animals—including the African clawed frog, the zebrafish, and the chicken—to reconstruct the melanopsin family tree. They found that most vertebrates carry two versions of melanopsin, one similar to that originally found in *Xenopus* and the other more similar to the human gene. Bellingham and colleagues called these two versions of melanopsin Opn4x, after *Xenopus*-like, and Opn4m, for mammal-like. They bolstered the case for two lineages of melanopsins by looking at the chromosomes that carry the different versions of melanopsin genes, and at the genes that sit alongside the melanopsin genes. Importantly, this also revealed that mammals have lost the Opn4x gene during their evolution, leaving them unusual among the vertebrates in having only one melanopsin.

With the revised lineage, it is clear that each version of melanopsin was fairly well conserved through evolution—except for the loss of Opn4x in the lineage leading to mammals. For both Opn4x and Opn4m, the sequences around the core region had been at least 66% conserved. This is significantly higher than the conservation estimates from

DOI: 10.1371/journal.pbio.0040268
Avoiding Punishment Is Its Own Reward

Liza Gross | DOI: 10.1371/journal.pbio.0040247

For my now-departed, wonderful old cat named Bear, life didn’t get any better than raw shrimp. Seeing the little white package emerge from the fridge always caught his attention, but what set him into high-shriek mode was the sound of shrimp being peeled under running water—he knew culinary bliss was at hand. Bear’s behavior was perfectly in keeping with the theory of reinforcement learning: through instrumental conditioning, animals learn to choose responses associated with producing favorable outcomes and avoiding unpleasant ones—typically by learning to associate two normally unrelated stimuli. The shrimp reward reinforced associations between stimulus (the sound of peeling and washing, rather than the sight of shrimp) and response (expectant wailing).

The flipside of reward learning, avoidance learning, doesn’t fit so neatly into the framework of reinforcement theories. Reinforcement theory predicts that behavior should rapidly disappear in the absence of explicit reinforcement. But studies show that once an animal manages to avoid punishment—for example, when a monkey learns to avoid a bitter drink by pressing a particular button—it may continue to perform the avoidance response even when it never experiences negative feedback again.

This apparent disconnect between avoidance learning and reinforcement theory could be resolved if avoiding punishment is itself a reward, a hypothesis that intrigued Hackjin Hong. This apparent disconnect between avoidance learning and reinforcement theory could be resolved if avoiding punishment is itself a reward, a hypothesis that intrigued Hackjin Hong.

Since the researchers found two distinct versions of melanopsin in three classes of vertebrates—fish, amphibians, and birds—this suggests the two versions split early in vertebrate evolution, before their ancestors came onto land, about 360 million years ago. Similarly, the analysis suggests that the lineage leading to mammals lost Opn4x early on, perhaps even before the separation of placental and marsupial mammals. Bellingham and colleagues also showed that the Opn4m of chickens functions as a sensory photopigment, as had been shown before for human and mouse melanopsins, confirming that the function of this gene has been conserved over evolutionary time.

So far, it’s not clear how the functions of the two versions of melanopsin differ. Maintaining separate cone opsin genes allows animals to sample different wavelengths of light and forms the basis of color vision. Could the two melanopsins underlie a similar quality for non-visual light detection? The researchers hope to answer this question by looking at the two melanopsin proteins of non-mammals in more depth. This will also reveal the implications for mammals of having only one melanopsin. Mammals also lost other photoreception proteins early in their evolution, leaving their color vision less sophisticated than that of birds, reptiles, fish, and amphibians. The loss of Opn4x reveals another way in which our experience of the light environment may be impoverished.

Bellingham J, Chaurasia SS, Melyan Z, Liu C, Cameron MA, et al. (2006) Evolution of melanopsin photoreceptors: Discovery and characterization of a new melanopsin gene in nonmammalian vertebrates. DOI: 10.1371/journal.pbio.0040254

Kim, Shinsuke Shimojo, and John O’Doherty. This possibility has been proposed before, but never tested. In a new study, Kim et al. investigated this question by scanning the brains of humans performing a simple instrumental conditioning task. A brain area called the medial orbitofrontal cortex (OFC) has been linked to reward-related stimuli, particularly when the reward involves money. The researchers reasoned that if avoidance learning and reward were equivalent, then the OFC should be activated in both contexts. If they are distinct cognitive processes, then each process should activate different regions.

Sixteen people participated in the study, during which they could either lose or win one dollar in an instrumental choice task. During the experimental trials, participants selected one of two fractal images presented on a screen. After a fractal was chosen, it became brighter, and four seconds later the participant got one of four types of feedback: reward (a picture of a dollar bill and the message, “You win $1!”), negative outcome (same image, with the text, “You lost $1!”), neutral (a scrambled bill with the text, “No change”), or nothing (a blank screen). During reward trials, the choice led to a high or low probability of reward (earning a dollar); during avoidance trials, the choice led to a high or low probability of avoiding a negative outcome (losing a dollar).

Over time, participants learned to choose fractals associated with a greater probability of reward and a lower probability of a negative outcome. And, as predicted, the medial OFC showed a higher response when participants chose an option that resulted in not losing the dollar or in winning it. Conversely, when participants’ choices resulted in negative outcomes—and when there was no reward offered—OFC activity declined. Compared to neutral trials, reward and avoidance events produced significantly greater brain activity, while negative outcomes and neutral events linked to no chance of reward resulted in significantly decreased activity. Kim et al. argue that these functional magnetic resonance imaging (fMRI) results “provide direct evidence” that avoiding bad outcomes and receiving a reward provoke a similar response in the medial OFC.
Ancient Protein Partners Take on Additional Roles in Multicellular Animals

Richard Robinson | DOI: 10.1371/journal.pbio.0040266

Over the long course of life’s history, the appearance of a new function in an organism may be accompanied by a new protein. But, more often, the work is done by an old one that adds a new role to its repertoire. Such proteins are likely to be found in a wide variety of organisms, reflecting their ancient lineage and continuing relevance. Proteins never act in isolation, of course; instead, they bind to one or more others to carry out their tasks. And so, if one member of a protein pair has taken on a new function, it’s a good bet the other may have done so as well. In a new study, Vlad Seitan, Tom Strachan, and colleagues show that two proteins, whose interactions in yeast help chromosomes divide, have counterparts in a full range of other organisms, including humans. And true to prediction, the proteins don’t just continue to play their old roles—in animals, they also appear to help guide multicellular development.

The focus of the study is a pair of yeast proteins, Scc2 and Scc4. Bound together, they load the protein complex cohesion onto chromosomes to link together sister chromatids, ensuring proper separation in mitosis. Scc2 has orthologs—proteins with similar structure sharing a common ancestor—in both fruit flies and humans, known respectively as Nipped-B and delangin. But, until very recently, orthologs of Scc4 have not been found outside of a few fungal species. The authors set out to find binding partners for Nipped-B and delangin. Using Nipped-B as the bait, they snagged the protein product of the fly gene CG4203. The human counterpart of this protein, called KIAA0892, bound to delangin. Because both CG4203 and KIAA0892 are related to a nematode protein called MAU-2, the authors dubbed them fly and human MAU-2. Each of these was about the same size as Scc4 and, using specialized bioinformatics approaches, they confirmed that the sequences of all three were related. Thus, Scc2 is to Scc4 as Nipped-B is to fly MAU-2, and delangin is to human MAU-2.

Up to this point, the only demonstrated functional similarity between Scc4 and the MAU-2s was their ability to bind their respective partners. To test whether human MAU-2 had a similar role in linking sister chromatids, the authors used RNA interference to diminish MAU-2 expression. When the level of MAU-2 was low, less cohesion was loaded onto the chromosomes, and sister chromatids prematurely separated, just as in yeast.

The expectation of reward also produced heightened activity in the medial and lateral OFC. To analyze the learning response during reward and avoidance trials, the researchers input the results of the behavioral experiments into a computational reinforcement model. As participants received rewards over the course of learning, those choices resulting in reward increased in value; by contrast, the value of choices resulting in bad outcomes decreased. As links between actions and their outcomes become clearer, the wisdom or folly of a choice also becomes clearer.

Avoiding negative outcomes and receiving rewards amount to the same thing for the brain: achieving a goal. Reward serves as an external signal that reinforces behavior associated with a positive outcome, Kim et al. explain, and punishment amounts to an intrinsic reward signal that reinforces actions linked to avoiding bad outcomes. With fMRI evidence connecting avoidance and reward circuits, researchers can now determine which neuron populations within the OFC contribute to the avoidance–reward response—and perhaps shed light on the neurobiological roots of pathological risk-seeking behavior.

Kim H, Shimojo S, O'Doherty JP (2006) Is avoiding an aversive outcome rewarding? Neural substrates of avoidance learning in the human brain. DOI: 10.1371/journal.pbio.0040233
In the Ant’s Garden, Fungal Parasites Are Closely Matched to Their Fungal Hosts

Richard Robinson | DOI: 10.1371/journal.pbio.0040259

Like all gardeners, fungus-growing ants risk losing their crop to pests. In particular, the cultivated fungi are parasitized by other fungi—of the species *Escovopsis*—which invade the fungal garden and consume the crop within. In virtually all cases, a single species of ant grows only a narrow range of genetically similar fungal cultivars, and these, in most cases, are parasitized by a few or only one type of *Escovopsis*.

While the ants do their best to protect their crop, it is important for the fungus itself to ward off the depredations of *Escovopsis*. Exactly how they do this is unknown. In a new study, Nicole Gerardo and colleagues demonstrate that different fungal cultivars have different abilities to ward off potential parasites; that successful parasites can best overcome the defenses of that cultivar they typically infect; and that host-switching by *Escovopsis* is likely inhibited by the genetically based specificity of these relationships.

The authors first showed that growth of the various *Escovopsis* types toward their preferred fungal cultivars is driven by a signal from the target. Yellow-spored *Escovopsis* grew faster toward its preferred host (cultivar A) than toward no target, or toward cultivar B, which it does not feed on. Brown-spored *Escovopsis*, which feeds on both A and B, grew faster toward either than toward no target. Neither the yellow nor the brown types did well against cultivar C, which is only distantly related to their normal hosts. In many cases, cultivar C kept these predators at bay for up to several months.

In some trials, however, C did not repel the yellow type and, in fact, in some trials, the yellow fungus grew faster toward C. This suggests genetic variability in the two fungal groups, which the authors confirmed. They showed that genetically similar *Escovopsis* strains were likely to be inhibited by the same C cultivar, and vice versa—genetically similar C cultivars were likely to inhibit a specific *Escovopsis* strain.

Over time, such close relationships, and the difficulty of overcoming non-host defenses, may inhibit host switching on the part of *Escovopsis*, and lead to synchronous speciation of the fungal hosts and parasites. On the other hand, the genetic variation that allowed some yellow *Escovopsis* to attack the C cultivars suggests that host switching remains a possibility among these fungi.

These results suggest that the ability of a specific *Escovopsis* type to parasitize a specific fungal cultivar is aided both by the chemical cues that attract the parasite and the close match between the host’s defensive weaknesses and the parasite’s strengths. The rapid growth this allows may help the parasite to stay ahead of the ant’s anti-fungal defensive mechanisms (like any good gardener, the ant farmers weed out the species they don’t want in their garden). More broadly, these results help clarify the variety of mechanisms involved in host–parasite relationships, which exist across all the domains of life.

Gerardo NM, Jacobs SR, Currie CR, Mueller UG (2006) Ancient host–pathogen associations maintained by specificity of chemotaxis and antibiosis. DOI: 10.1371/journal.pbio.0040235

The Red Queen Gets a New Lease on Life

Liza Gross | DOI: 10.1371/journal.pbio.0040285

Why does sex exist? A long-popular view holds that sexual reproduction creates new gene combinations that help the next generation resist rapidly co-evolving parasites. Each species constantly changes to achieve the same result—evolutionary advantage—prompting evolutionary biologists to dub this hypothesis the Red Queen (who tells Alice in *Through the Looking Glass* “it takes all the running you can do, to keep in the same place”).

Recent theoretical studies have challenged the generality of the Red Queen hypothesis, suggesting that even though parasites can exert selection pressures that favor sex under some conditions, more often they select...
enhance genetic mixing and alleles between gene variants (or alleles) that species interactions should create links and maintenance of sex, then these evolution underlies the evolution and similarity selection.

Agrawal explains, to the extent that maternal transmission occurs, hosts will be subject to both genotypic and similarity selection.

If the Red Queen hypothesis is true, and host–parasite co-evolution underlies the evolution and maintenance of sex, then these species interactions should create links between gene variants (or alleles) that enhance genetic mixing and alleles related to fitness. (The alleles that influence genetic mixing are called modifier alleles, because they influence the degree of investment into sexual rather than asexual reproduction.)

Agrawal first determined how a modifier allele evolves under different scenarios involving genotypic and similarity selection. He then evaluated the extent of genotypic and similarity selection produced by host–parasite co-evolution, and showed how the likelihood of maternal transmission affects whether parasites select for or against sex. He found that even though similarity selection has a much weaker effect than genotypic selection on fitness, it can exert a powerful force on the evolution of modifier alleles (and thus sex). Even a small chance of maternal transmission can lead to parasite selection for sex, Agrawal explains, because similarity selection affects genetic associations between mother and offspring, which tend to be strong (as opposed to genetic associations within offspring, which tend to be weaker).

Previous models have shown that sex is favored under very limited conditions in large, randomly breeding populations because genetic mixing tends to break down beneficial gene combinations produced by selection, which presumably enhance fitness. By incorporating the fitness effects of similarity selection, Agrawal could examine similarity selection’s potential impacts on the evolution of modifier alleles independent of its fitness effects—and discover that parasites are “much more likely to favor sex.” The model predicts that this is most likely to occur when parasites are directly transmitted from mother to offspring, virulence is low, and infection rates are high (otherwise, too few offspring are produced by infected mothers).

While Agrawal doesn’t argue that parasites fully explain why sex evolved, his results show that accounting for real-world transmission scenarios puts the ball squarely back in the Red Queen’s court. Researchers can use his model to study the evolution of sex under a wide range of scenarios, such as when individual fitness depends on kin or other social groups.

Agrawal AF (2006) Similarity selection and the evolution of sex: Revisiting the Red Queen. DOI: 10.1371/journal.pbio.0040265

**Wnt Sets the Stage for Spinal Cord Patterning in the Chick**

*Françoise Chanut | DOI: 10.1371/journal.pbio.0040280*

The spinal cord and brain harbor a variety of motor neurons that innervate limbs, thorax, neck, or face. Different classes of motor neurons reside at different locations along the head-to-tail (rostrocaudal) axis of the nervous system, reflecting a patterning that arises early in embryonic development, when the nervous system is but a simple tube. Indeed, different sections of the neural tube begin expressing distinct subsets of genes thought to govern cell fate, such as members of the *Hox* gene family, shortly after the tube forms. The question is how this patterned gene expression arises. Ulrika Nordström, Thomas Edlund, and their colleagues have tackled this problem in the chick embryo. They report that rostrocaudal patterning of the spinal cord and hindbrain is under the influence of the signaling molecule Wnt. Other signals refine Wnt patterning and create distinct sections in which different types of motor neurons eventually differentiate.

The embryonic nervous system of vertebrates is shaped by signals coming from surrounding tissues, in particular from the mesoderm, which gives rise to skeletal bones and muscles. The mesodermal signal fibroblast growth factor (FGF), for instance, imposes caudal fates on the developing nervous system, whereas Wnt and FGF in combination pattern the middle portions of the prospective brain. Since Wnt remains abundant in regions where the spinal cord will start to grow behind the nascent brain, Nordström and her colleagues reasoned that it might also pattern the posterior regions of the embryo’s nervous system.

The researchers cut out small fragments (called “explants”) of prospective neural tissue from chick embryos that had reached different developmental stages and observed the explants’ development in culture. At developmental stage 4, the chick embryo is a pin-head-size disc of cells that lies atop the yolk. Its most prominent feature is the primitive streak,
Coronary disease has long been considered an affliction of the affluent, a lifestyle disease caused by eating unhealthy foods, drinking and smoking too much, and not getting enough exercise. But a recent study of the global burden of disease, published in *The Lancet*, found that heart disease is the number one killer in rich and poor countries alike. The disease is so deadly partly because the human heart, unlike the skin or liver, can’t repair itself. Damaged heart tissue is replaced with scar tissue instead of healthy cells. Cardiomyocytes, the heart’s structural cells, sometimes bulk up to replace lost cardiac cells, a response that can lead to cardiac arrest.

Not all hearts are so vulnerable to insults. In a 2002 study, Mark Keating and his colleagues showed that zebrafish, which can grow new spinal cords, retinas, and fins, can also regenerate heart tissue. And now, Ching-Ling Lien and colleagues, in a new study led by Keating, use microarray analysis to reveal the molecular signals underlying this regenerative capacity. The researchers identified sets of wound-healing genes and growth factors with temporally distinct expression patterns and show that regeneration relies on platelet-derived growth factor (PDGF), a regulator of cell proliferation and development.

In the 2002 study, Keating’s group outlined the course of zebrafish heart regeneration. After an initial blood clot forms to stanch the bleeding, a more substantial fibrin clot forms a few days later. Nearby cardiomyocytes enter the cell cycle (triggering DNA synthesis and proliferation) about seven days after amputation to repair damaged heart tissue. By around 30 days, new cardiomyocytes have replaced lost tissue, and by two months, regeneration is complete.

To find potential drivers of heart regeneration, Lien et al. focused on the initial stages of regeneration. Following real and sham surgical amputations (the sham procedure stops just short of amputation to serve as a control), regenerating hearts were removed three, seven, and 14 days after the operation, and gene transcripts were extracted from the tissue. Microarray analysis revealed 662 transcripts with noticeably different expression patterns (increased or reduced activity) in at least one of the three time points.

The researchers classified the 662 genes into functional categories and expression patterns. Genes involved in the wound or inflammatory response were expressed early and peaked at three days, likely drawing inflammatory cells to the wound site. Starting from three days, several growth factors and secreted molecules, some associated with cardiac arrest, are expressed. These include members of the Wnt signaling pathway, which is crucial for embryonic development, and is necessary for the neural tube to adopt the posterior hindbrain. The researchers propose that, during normal development, early Wnt signaling steers the growing neural tube toward posterior fates, while retinoic acid and FGF later subdivide it into smaller rostrocaudal domains from which distinct motor neurons emerge.

**Nordström U, Maier E, Jessell TM, Edlund T (2006) An early role for Wnt signaling in specifying neural patterns of Cdx and Hox gene expression and motor neuron subtype identity. DOI: 10.1371/journal.pbio.0040252**

**Regenerating Zebrafish Hearts Reveal the Molecular Agents of Repair**

*Liza Gross* | DOI: 10.1371/journal.pbio.0040281

...and the presence of an inhibitor of Wnt signaling, the explants failed to turn on *Krox20* and the *Hox* genes, and instead expressed *Otx2*, a marker of the forebrain. Therefore, Wnt signaling appears necessary for the neural tube to adopt the posterior characteristics of hindbrain and spinal cord.

Missing from the cultured explants, however, were cells expressing a combination of *Hoxb8* and *Hoxb4*, which mark the thoracic and neck regions of the spinal cord, or *Hoxb4* alone, which marks the posterior hindbrain. By contrast, these cells appeared readily in cultured explants taken from stage 8 embryos. By this stage, mesoderm has crept through the backward-sliding Hensen’s node to lie under the elongating neural tube, where it begins to organize into somites, the precursors of vertebrae and ribs. The researchers reasoned that retinoic acid, a signal secreted by somites, might combine with Wnt to specify hindbrain and anterior spinal cord fates.

Adding retinoic acid to cultures of stage 4 explants confirmed this hypothesis. Retinoic acid suppressed the expression of *Krox20* and *Hoxc9*, while inducing the appearance of cells expressing *Hoxb8* and *Hoxb4*, or *Hoxb4* alone. If the cultures also contained FGF, which normally diffuses from the primitive streak, the explants turned mostly into *Hoxb8/b4*-expressing cells. Culturing stage 4 explants from the forebrain region in the presence of various combinations of Wnt with retinoic acid or FGF also led to the appearance of distinct rostrocaudal subsets of spinal cord markers.

The researchers propose that, during normal development, early Wnt signaling steers the growing neural tube toward posterior fates, while retinoic acid and FGF later subdivide it into smaller rostrocaudal domains from which distinct motor neurons emerge.
with wound healing, also showed increased expression. By day 7, genes involved in tissue remodeling took over, with increased activity lasting a week.

By monitoring gene expression of a subset of growth factors and other secreted molecules within the regenerating heart, Lien et al. confirmed that their activity was restricted to specific times and sites in and around the wound and heart muscle. A crucial mediator of cell proliferation, PDGF-A had high expression levels at day 7. It often forms complexes with PDGF-B, but expression levels at day 7. It often forms complexes with PDGF-B, but since the zebrafish version of the gene encoding this protein hadn’t been sequenced yet, Lien et al. had to first isolate it from the tissue and sequence it before determining its expression pattern. As it happened, PDGF-B was also active at day 7—the same time that cardiomyocytes begin DNA replication. Working with cultured cardiomyocytes, the researchers demonstrated that PDGF signaling induces DNA synthesis in the cells and is required for cardiomyocyte proliferation during heart regeneration.

This study demonstrates the value of using microarrays to home in on likely mediators of regeneration at different stages of the process. With their list of candidate genes, Lien et al. plan to use more-targeted techniques to examine the genes’ functions and to more fully investigate the role of PDGF signaling. And with the genetically pliable zebrafish as a model system, researchers will try to piece together the molecular program of heart regeneration, with an eye toward molecular therapies that could mend the human heart.

Lien CL, Schebesta M, Makino S, Weber GJ, Keating MT (2006) Gene expression analysis of zebrafish heart regeneration. DOI: 10.1371/journal.pbio.0040260

Keeping Things under Control at the Neuromuscular Junction

Mary Hoff  |  DOI: 10.1371/journal.pbio.0040289

In vertebrates, neurons signal muscles to contract by releasing a neurotransmitter, acetylcholine, into the intercellular space. They do so by fusing spherical acetylcholine-filled packages with the plasma membrane. But what controls when—and how many—such packages, known as synaptic vesicles, release their contents? A protein called tomosyn is thought to play a key role in keeping the process under control. But until now its mode of action was unknown.

To clarify tomosyn’s role in regulating acetylcholine release at the neuromuscular junction, Elena O. Gracheva, Janet E. Richmond, and colleagues enlisted the help of Caenorhabditis elegans, a salt-grain-sized nematode that, because of its simple nervous system and tractable genetics, is widely used to study how neurons work. C. elegans has a gene, tom-1, that makes a class of proteins, TOM-1, similar to vertebrate tomosyn. After discovering that C. elegans TOM-1 is biochemically very similar to tomosyn in vertebrates, the researchers decided to use the nematode as a model for exploring vertebrate tomosyn function.

They began by measuring traits of the nerve-to-muscle signal in tom-1 mutants that are unable to properly produce TOM-1. They found that the evoked release of acetylcholine onto the muscle was enhanced in tom-1 mutants relative to non-mutant (wild-type) organisms, due to a prolonged evoked response. Other studies have shown that tomosyn is able to block the formation of a protein complex called the SNARE complex, known to mediate vesicle release. Could this be how tomosyn regulates synaptic transmission? Before they could conclude so, the researchers had some alternative explanations to discount. By comparing post-synaptic electrophysiological activity in wild-type and mutant strains, they showed that the effects observed in the tom-1 mutants were not due to changes in post-synaptic receptor kinetics. By comparing the morphology of neurons in wild-type and mutant strains, they also showed the effects were not due to altered neuronal connectivity.

And by comparing the number and distribution of neuromuscular synapses in the two (using a technique called immunolabeling), they demonstrated the moderating effect of tomosyn is not due to changes in how synapses develop, either.

Next they tested whether the tom-1 mutant trait could be reversed by selectively expressing TOM-1 in cholinergic neurons (acetylcholine-producing neurons). They found the nerve-to-muscle signal to be less prominent in this situation than in either wild-type or tom-1 strains, while the duration of the response evoked in the muscle was like that of wild-type worms. The conclusion: TOM-1 regulates synaptic transmission on the nerve side, rather than on the muscle side, of the synapse.

With the hypothesis that tomosyn acts on the vesicle release part of signal transmission strengthened by these findings, the researchers decided to look more closely at the vesicles themselves. Electron microscopic examination of synapses in wild-type and mutant strains showed the number of vesicles did not differ but that vesicle distribution did, with tom-1 mutants having more vesicles in contact with the plasma membrane and a broader distribution along the membrane. In addition, the mutant worms whose cholinergic function had been restored through selective TOM-1 expression had fewer contacting vesicles and less distributed vesicles in their cholinergic synapses.

An important part of the vesicle-signaling process at the neuromuscular junction is the preparation of vesicles for release, a process known as
“priming.” Previous research has shown that synapses in a C. elegans mutant known as unc-13(s69), which has priming problems, have few vesicles in contact with the plasma membrane and do not release acetylcholine. This suggests that the vesicles lined up on the membrane in the tom-1 mutants represent a superabundance of primed vesicles. To test this hypothesis, the researchers produced tom-1 unc-13(s69) double mutants. As they predicted, these double mutants had more contacting vesicles than unc-13(s69) alone and restored acetylcholine release. The researchers concluded that TOM-1’s role in moderating signal transmission at the neuromuscular junction occurs by limiting the number of vesicles primed to release acetylcholine into the intercellular space.

Gracheva EO, Burdina AO, Holgado AM, Berthelot-Grosjean M, Ackley BD, et al. (2006) Tomosyn inhibits synaptic vesicle priming in Caenorhabditis elegans. DOI: 10.1371/journal.pbio.0040261

Math and Fossils Resolve a Debate on Dinosaur Metabolism

Liza Gross  |  DOI: 10.1371/journal.pbio.0040255

Of the many mysteries surrounding the life history of dinosaurs, one of the more enduring is how such gigantic organisms—some reaching 42 feet tall and weighing 90 tons—regulated their body temperature. For many years, scientists had assumed that dinosaurs, which evolved from reptiles, were also cold blooded (ectotherms), with a slow metabolism that required the sun’s heat to thermoregulate. But, in the late 1960s, the notion emerged that dinosaurs, like mammals and birds, might have been warm blooded (endotherms) with relatively constant, high body temperatures that were internally regulated like their avian descendants (and mammals).

Still others argued that while most dinosaurs had a metabolism similar to contemporary reptiles, the large dinosaurs managed a higher, more constant body temperature through thermal inertia, which is how modern alligators, Galapagos tortoises, and Komodo dragons retain heat. Thermal inertia allows the body to approach homeothermy, or constant body temperature, when the ratio of body mass to surface area is high enough. If this “inertial homeothermy” hypothesis is correct, dinosaur body temperature should increase with body size.

In a new study, James Gillooly, Andrew Allen, and Eric Charnov revisit—and resolve—this debate. The researchers used a model that provided estimates of dinosaur body temperature based on developmental growth trajectories inferred from juvenile and adult fossil bones of the same species. The model predicts that dinosaur body temperature did increase with body mass, and that large dinosaurs had body temperatures similar to those of modern birds and mammals, while smaller dinosaurs’ temperatures were more like contemporary reptiles. These results suggest that the largest dinosaurs (but not the smaller ones) had relatively constant body temperatures maintained through thermal inertia.

Gillooly et al. compiled data from eight dinosaur species from the early Jurassic and late Cretaceous periods that ranged in size from 30 pounds to 28 tons. The growth trajectories, taken from the published research papers, were determined by using bone histology (microscopic study) and body size estimates to estimate the maximum growth rate and mass at the time of maximum growth. The recent availability of these data, the researchers explain, along with advances in understanding how body size and temperature affect growth, allowed them to use a novel mathematical model to estimate dinosaur body temperatures. The researchers modified the model to estimate the body temperature of each dinosaur species, based on its estimated maximum growth rate and mass at the time of maximum growth. The model shows that body temperature increases with body size for seven dinosaur species.

The model shows that dinosaur body temperature increased with body size, from roughly 77 °F at 26 pounds to 105.8 °F at 14 tons. These results, the researchers explain, suggest that the body temperatures of the smaller dinosaurs (77 °F) were close to the environmental temperature—just as occurs for modern smaller reptiles—which meant they acquired heat from external sources (in addition to the internal heat generated by metabolism). The results also suggest that body temperature rose as an individual dinosaur grew, increasing by about 37.4 °F for species weighing about 661 pounds as adults and nearly 68 °F for those reaching about 27 tons (Apatosaurus excelsus). Predicted body temperature for the largest dinosaur (Sauroposeidon proteles at about 60 tons) was about 118 °F—just past the limit for most animals, suggesting that body temperature may have prevented dinosaurs from becoming even bigger.

Gillooly et al. demonstrate the validity of these results by showing that the model successfully predicts documented increases in body temperature with size for existing crocodiles. Altogether, these results indicate that dinosaurs were reptiles and that their body temperature increased with body size—providing strong evidence for the inertial homeothermy hypothesis.

Gillooly JF, Allen AP, Charnov EL (2006) Dinosaur fossils predict body temperatures. DOI: 10.1371/journal.pbio.0040248
Life exists in an impressive variety of forms, but the processes responsible for generating all that variation typically take far longer than the average biologist’s career. Luckily, many single-celled organisms with short generation times and prolific reproductive capacity can be easily manipulated to study the mutations associated with adapting to environmental changes.

In a new study, Ayellet Segrè, Andrew Murray, and Jun-Yi Leu describe a novel way to map evolved traits to their chromosomal location, using a genomic technique called linkage analysis. Adaptive mutations are detected based on their proximity (linkage) to neutral genetic markers (called DNA polymorphisms) observed in the ancestor. When the researchers applied their method to a parallel evolution experiment—in which four yeast strains independently adapted to alternating carbon sources—they found that each strain had acquired adaptive mutations in the same gene.

Yeast can be haploid (carry a single genome copy) and diploid (carry two copies). To generate genomic DNA for their mapping method, the researchers mated a “target” haploid yeast strain (expressing a specific trait) with a haploid “reference” strain (that lacks the trait and differs from the target strain at thousands of polymorphic sites) to create a hybrid diploid. Meiosis (cell division that turns diploid cells into haploid cells) was induced to produce a diverse assembly of recombinant offspring called segregants. From the progeny, one pool of segregants was selected that expresses the trait and a randomly collected pool served as a control.

Segrè et al. identified polymorphic sites that differed between the target and reference strains by placing DNA from each strain onto specialized microarrays called high-density oligonucleotide arrays. They chose polymorphic sites based on which oligonucleotides bound more strongly (hybridized) to target DNA than to reference DNA. (The genomic DNA is fragmented, denatured, and labeled with a fluorescent dye. Fragments that are not polymorphic relative to their complementary feature on the array light up more than polymorphic fragments.)

The method maps polymorphisms that segregate with the trait based on which oligonucleotides hybridize more strongly to DNA from trait-expressing progeny than from control progeny. After testing their method on two strains with sequenced genomes, Segrè et al. identified over 10,000 polymorphisms between their target and reference strains, called single feature polymorphisms, or SFPs—providing dense genome coverage for their linkage analysis map. (Each SFP corresponds to one oligonucleotide.) Hybridization intensities were converted into a linkage map score (LMS), which estimates the probability that the chromosomal location is linked to a selected trait.

This approach was then used to map five genes with known chromosomal locations in pools of segregants created from crossing a target strain (that can make the amino acid lysine and resist the toxicity of four drugs) and a reference strain (that lacks all five genes), and selecting for segregants that could make lysine or that could grow in a medium laced with toxic drugs. Among the highest LMS were the chromosome positions containing the five trait-related genes.

Having shown that their technique can map known genetic markers, they tested it on novel mutations underlying a trait they had evolved in laboratory yeast populations. They used four yeast populations alternately grown in media containing glucose or galactose (yeast typically grow faster on glucose) for
What transforms a ball of undifferentiated cells into an organism with a nervous system, digestive tract, and other specialized body parts? Among the proteins that play an important role is one with the unlikely name of hedgehog (Hh). When Hh attaches to a transmembrane protein known as patched (Ptch1), it initiates a series of molecular interactions that lead to activation of the transcription factor Gli (for “glioma associated”) and the onset of key events in embryonic differentiation.

We know this process involves freeing a second transmembrane protein, smoothened (Smo), from inhibition. But how do Hh and Ptch1 accomplish this? Scientists would like to know because the ability of this signaling pathway to function properly makes the difference between normal development and devastating abnormalities—and because the pathway is also implicated in tumor growth.

Maarten F. Bijlsma, Maikel P. Peppelenbosch, and colleagues began their attempt to find out by noting that previous studies show that enzymes used to make cholesterol are involved in the pathway; that Ptch1 and Smo don’t necessarily bond to each other; that Ptch1 looks like other proteins that pump molecules from one side of the cell membrane to the other; and that cholesterol-like molecules can inhibit the pathway. Based on that information, the researchers hypothesized that when—and only when—Ptch1 is unencumbered by Hh, it pumps a cholesterol-like molecule into the extracellular space, where it inhibits Smo.

Inhibiting Hedgehog: New Insights into a Developmentally Important Signaling Pathway

Mary Hoff  |  DOI: 10.1371/journal.pbio.0040258

To test this, the researchers developed an experimental system made up of fibroblasts modified to luminesce when Gli is active (called reporter cells), and to overexpress various combinations of Ptch1, Smo, and Gli. When they mixed reporter cells overexpressing Smo with cells overexpressing Ptch1, Gli activation in the reporter cells was reduced. When they mixed them instead with cells in which Ptch1-producing genes were silenced, Gli activation increased. After performing additional tests to eliminate alternative explanations, the team concluded that Ptch1 inhibits Smo through an intermediary, and that the intermediary molecule can exert its influence between individual cells.

The researchers next exposed reporter cells to a medium that had contained Ptch1-overexpressing cells, and found Gli activation to be strongly inhibited. However, when they exposed reporter cells to a serum-free, Ptch-conditioned medium, they found no inhibition. Since serum-free medium doesn’t contain lipoproteins, they concluded that a lipoprotein is involved. Further tests suggested that the lipoprotein acts by helping transport a 3β-hydroxysteroid involved in the pathway inhibition.

Interestingly, the researchers noted that certain people with Hh signaling problems have elevated levels of a particular hydroxysteroid, 7-dehydrocholesterol (7-DHC). This led them to test the link between 7-DHC and Gli activity in mouse cells, which in turn led to the conclusion that 7-DHC indeed participates in Ptch1’s inhibition of Smo.

But is the Smo-inhibiting molecule actually 7-DHC or a compound derived from 7-DHC? When the researchers exposed medium from 7-DHC-producing mouse cells to ultraviolet radiation—which changes 7-DHC into vitamin D3—or used vitamin D3 in place of 7-DHC, Gli activity in mouse cells was strongly inhibited. Since serum-free medium doesn’t contain lipoproteins, these findings suggest that vitamin D3 is involved in the pathway inhibition.
7-DHC, Hh pathway inhibition was even stronger. They also showed that vitamin D3 binds to Smo, and that it inhibits the Hh pathway in live zebrafish embryos.

Putting it all together, the researchers concluded that when Hh isn’t present, Ptch1 pumps (pro)-vitamin D3 (i.e., either 7-DHC or vitamin D3) into the extracellular space, where the hydroxysteroid grabs onto Smo, inhibiting Gli activation. When Hh binds to Ptch1, the pump grinds to a halt, Smo is freed of inhibition, and transcription of developmentally important genes kicks in.

This new knowledge of the Hh signaling pathway shows how some cells can affect neighboring cells’ development, and it helps explain some of the problems associated with mutations affecting cholesterol biosynthesis. Because the Hh pathway is linked with certain cancers, it also has implications for tumor development. Additional work is now underway to see whether this new understanding of Ptch1’s intercellular inhibition of Smo can be applied to help suppress tumor growth.

Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F, et al. (2006) Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. DOI: 10.1371/journal.pbio.0040232