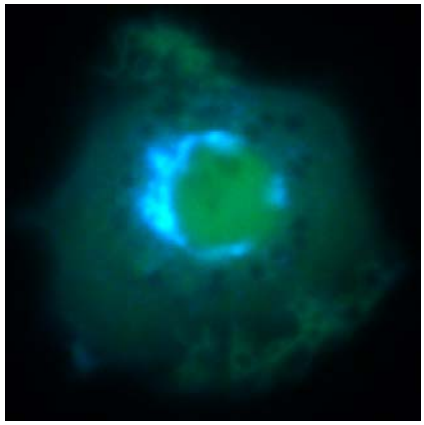
Inconspicuous Consumption: Uncovering the Molecular Pathways behind Phagocytosis

Mason Inman | DOI: 10.1371/journal.pbio.0040190

Eating at an Ethiopian restaurant might give one an appreciation for the process of phagocytosis, a widespread method immune cells use to ingest particles. To dine using injera, the spongy Ethiopian flatbread, you form a pocket of bread around the food that you pinch off and, with practice, stuff cleanly into your mouth. Similarly, when cells ingest small particles, viruses, or other smaller cells by phagocytosis, they extend protrusions called pseudopods around their target. The pseudopods then seal around the object, and a bit of the cell membrane that encases the object buds off and travels inside the cell.

Figuring out which molecules are involved in coordinating the fast-moving, dynamic process of phagocytosis has been difficult. But using a sensitive, high-resolution technique called fluorescence resonance energy transfer (FRET), a new study reveals the patterns of protein activities that help regulate phagocytosis. Peter Beemiller, Adam Hoppe, and Joel Swanson show that two stages of phagocytosis—the extension of pseudopods and the closure around an object—each require activation of specific proteins, members of the ADP ribosylation factor (ARF) family, which are major regulators of the budding of vesicles, or membrane-bound sacs, from internal organelles. The researchers also found that an enzyme called phosphatidylinositol-3′-kinase (PI-3K) plays a key role in organizing these two stages of phagocytosis.

Key to the discovery was a technique called FRET. Though the technique was first described nearly 50 years ago, it has recently been greatly refined and has experienced a renaissance. The technique relies on the ability of one fluorescent molecule to excite another fluorescent molecule, which emits light when the two molecules are very close to each other. Thus, when exciting the first fluorescent molecule lights up the second one, it signals that the two fluorescent molecules are within tens of angstroms—practically on top of each other.



DOI: 10.1371/journal.pbio.0040190.g001

Fluorescent proteins localize the guanosine 5'-triphosphate hydrolase ARF in the Golgi apparatus of a living macrophage. FRET studies revealed ARF activation in the Golgi and in the formation of phagosomes.

To use FRET for picking apart the pathways involved in phagocytosis, the researchers made mouse phagocytes that expressed engineered proteins—versions of ADP ribosylation factors (ARFs) 1 and 6 had fluorescent protein attached, and fluorescent marker proteins that would bind to either ARF1 or ARF6, when they had been activated inside the cell. Binding of the activated fluorescent ARFs to the fluorescent markers could be detected via FRET. Thus, the researchers could see when and where ARF1 or ARF6 were active in the cell during phagocytosis.

The researchers found that pseudopods extending around a particle activated ARF6 at their tips. Then as the cell more fully enveloped a particle, ARF6 turned off, and activated ARF1 appeared throughout the pseudopod until it closed around the particle. The discovery of a role for ARF1 was unexpected, since previous studies suggested this protein is not involved in phagocytosis. Also, the researchers found that inhibiting PI-3K prevented cells from switching between these two phases of phagocytosis, and caused them to stop halfway without having closed around the particles.

It's still not entirely clear what role each of the ARFs plays. ARF6 may regulate how the cell supplies more membranes to the area of phagocytosis, so that the cell can expand its surface area there and engulf particles. ARF1, on the other hand, plays a key role in protein trafficking, and so may regulate which proteins migrate to the site of phagocytosis. But finding that phagocytosis has distinct phases, regulated by phospholipids in the membrane, is an important step toward sorting out how thousands of receptors can be coordinated in time and space to make a phagosome.

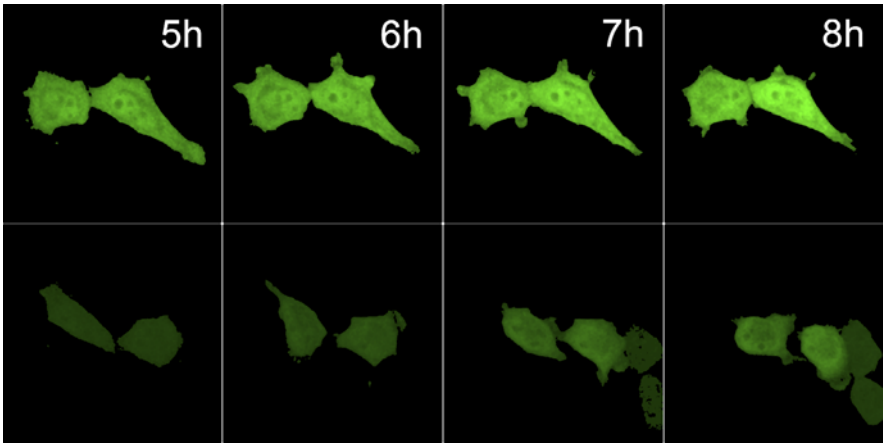
Beemiller P, Hoppe AD, Swanson JA (2006) A phosphatidylinositol-3-kinase-dependent signal transition regulates ARF1 and ARF6 during Fcγ receptor-mediated phagocytosis. DOI: 10.1371/journal.pbio.0040162

More GC Means More RNA

Richard Robinson | DOI: 10.1371/journal.pbio.0040206

The genetic code that dictates the translation of RNA triplets into amino acids is rife with synonyms. Both CUG and CUA encode the amino acid leucine, for instance, and CCC and CCU encode phenylalanine. Each member of these synonymous pairs works equally well—the use of any particular synonym has no effect on the incorporation of its corresponding amino acid. But in mammalian genes, there is a surprising cumulative effect—genes with a greater proportion of third-position Gs or Cs are expressed more than genes with third-position As or Us. This effect is so pronounced that some researchers use the GC content of a multigene segment of a chromosome as a rough measure of its protein-producing activity.

There is a long path from gene to protein, and it is unclear at what step along that path the GC versus AU difference exerts its influence. A



DOI: 10.1371/journal.pbio.0040206.g001

Cultured human cells produce ten times more green fluorescent protein from a GC-rich gene (top row) than from a synonymous GC-poor gene (bottom row).

new study by Grzegorz Kudla, Leszek Lipinski, and colleagues show that the difference is neither in translation of RNA into protein nor in RNA stability, but in production, or transcription, of RNA from the DNA template.

The authors began by comparing two genes that encode protein-stabilizing heat shock proteins, Hsp70 and Hsc70. The coding regions of the two genes are similar in length, and the proteins are largely similar in sequence. The "GC3" for Hsp70 is 92%, meaning it uses a G or C at 92% of its third positions, while the GC3 for Hsc70 is 46%. Within the cell, however, the genes also differ in their location in the chromosome, their promoters (which bind the RNA-synthesizing machinery), and other important determinants of gene expression. To control for these potentially confounding factors, the authors introduced the genes for Hsp70 and Hsc70 into cells in chromosome-independent vectors. They found the GC-rich gene produced ten times as much protein as the GC-poor one, an effect that was independent of the type of cell. This effect was not due to the rate of protein synthesis, which was the same when equal amounts of the messenger RNA for each gene was present. Neither was it due to differences in RNA stability, which was also similar for the two genes. Instead, the difference was due to production of RNA.

This effect was not limited to the heat shock proteins. Similar differences were seen with GC-rich and -poor versions of genes for the mammalian immune system protein interleukin-2, as well as for green fluorescent protein, from the very nonmammalian jellyfish. Neither was it limited to the extrachromosomal position of the introduced gene: GC-rich genes integrated into the chromosome outperformed GC-poor ones in exactly the same spot.

This study does not address the "how" of the GC advantage—the mechanism by which GC-rich genes are transcribed more than GC-poor ones. But it does identify the "when," which should speed research into the mechanism, and may ultimately help illuminate the "why"—the evolutionary reasons behind this difference. These results also confirm the utility of increasing GC content of genes used for medical therapies, where increased expression can mean the difference between life and death, and for biotechnology applications, where more protein means more product and more money.

Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M (2006) High guanine and cytosine content increases mRNA levels in mammalian cells. DOI: 10.1371/journal.pbio.0040180

Plants Tell Caterpillars When It's Safe to Forage

Liza Gross | DOI: 10.1371/journal.pbio.0040194

Light and temperature exert significant control over the daily lives of plants and animals. Many nocturnal species feed during the cover of night to escape a bevy of potential predators that hunt during the day. The effect of photoperiod (the 24-hour daylight cycle) is so great that zoos can trick nocturnal animals into becoming active for visitors simply by keeping their exhibit dark in the daytime and illuminating it at night.

But the world is filled with cues that could influence the daily feeding patterns of an organism. Many plants, for example, respond to foraging damage by releasing specialized chemical signals—volatile organic compounds that evaporate in the air—that attract the forager's natural enemies. This strategy is obviously no use against a cow, but proves effective when the offender is a caterpillar and the summoned predator is a wasp. Just how much control such biotic factors exert over a forager's daily routine has remained an open question. But in a new study, Kaori Shiojiri, Rika Ozawa, and Junji Takabayashi show that plant signals can indeed regulate herbivore behavior.

DOI: 10.1371/journal.pbio.0040194.g001

Plant volatiles released by corn plants may help the caterpillar *M. separata* avoid predators.

When the larvae of beet armyworms *(Spodoptera exigua)* feed on corn, the plant releases volatile compounds that act as a magnet for parasitic wasps *(Cotesia marginiventris)*, which deposit their eggs in the larvae. Production of volatile chemicals increases during the day (when wasps are active) and decreases at night, suggesting that variations in production might affect the daily activity patterns of foraging larvae, with low production sending the signal that the coast is clear.

To test this hypothesis, Shiojiri et al. exposed larvae of a corn-munching caterpillar, *Mythimna separata*, to volatile compounds from corn and varied the light and dark conditions for both corn and insect. Corn infested with *M. separata* releases volatiles that attract parasitic wasps *(C. kariyai)*. Previous work had established that *M. separata* is nocturnal, based on experimental observations of the larvae's daily feeding and hiding behavior on corn plants, but the impact of variable volatile production had not been examined.

In this study, the researchers separated the effects of photoperiod from that of host plant volatiles to tease out their relative contributions to caterpillar behavior. First, they tested the effects of light. If larvae are diurnal, they should hide in "shelters" fashioned out of filter paper attached to the plastic cups they were kept in. When larvae were fed an artificial diet, however, different light conditions produced no changes in their hiding behavior.

But introducing plants changed larvae behavior under both day and night conditions. Six pots of three uninfested corn plants—plants that had never been grazed—were placed around the cups of larvae. After eight hours, about 20% more larvae went into hiding when the lights were on and plants were added. And when plants were introduced under dark conditions, about 30% *less* larvae were found hiding than were found in the dark without plants.

To test the effect of plant volatiles directly, the researchers exposed larvae—some in the light and some in the dark—to a flow of volatiles collected from both uninfested and infested corn plants in light and dark conditions. When larvae in the dark were exposed to volatiles from uninfested plants, they hid in far greater numbers when the volatiles came from plants in the light than when they came from plants in the dark. And when larvae were in the light, far more hid when exposed to volatiles taken from plants in the light. Larvae responded similarly to volatiles taken from infested plants, though volatiles from infested plants in the light sent even more larvae into hiding.

These results demonstrate that it is not light that's controlling larval diurnal and nocturnal activity but volatiles released by the corn. Volatile compounds released during the day encourage hiding while those released at night indicate that it's safe to come out and eat. Just as parasitic wasps use plant volatiles to home in on potential victims, caterpillars use variations in their host plant's volatile production to reduce the risk of unpleasant encounters with wasps. Now that they've established volatiles' importance in influencing foraging behavior, the researchers plan to determine which compounds are responsible—and just how common insect–plant communication may be.

Shiojiri K, Ozawa R, Takabayashi J (2006) Plant volatiles, rather than light, determine the nocturnal behavior of a caterpillar. DOI: 10.1371/journal.pbio.0040164

A New View on Lyme Disease: Rodents Hold the Key to Annual Risk

Liza Gross | DOI: 10.1371/journal.pbio.0040182

Any kid who spent summers playing in the woods knew Mom wouldn't let you back in the house without a head-to-toe search for ticks—vectors for a wide range of pathogens throughout the world's temperate regions. In the United States, Rocky Mountain spotted fever was the main concern until 1975, when Lyme disease was found in Connecticut. Since then, incidence has skyrocketed from 497 cases reported in 1982 (the first year national statistics were collected) to a record 23,763 in 2002.

Lyme disease, like other zoonoses, is transmitted by a vector that picks up the pathogen during a blood meal from a vertebrate host. In the eastern and central United States, the spirochete bacterium *Borrelia burgdorferi* infects blacklegged ticks, *Ixodes scapularis*, which feed on a wide variety of birds, lizards, and mammals, including mice, deer, and humans. Since human risk is a function of the prevalence of infection among vectors, outbreak prevention depends in part on understanding what controls infection rates among the agents of transmission.

DOI: 10.1371/journal.pbio.0040182.g001

The blacklegged tick *(Ixodes scapularis)*, the primary vector for Lyme disease in the central and eastern United States.

In a new study, Richard Ostfeld, Felicia Keesing, and colleagues examined the ecological determinants of Lyme disease over a 13-year period in southeastern New York, a hot zone for the disease. Combining field data with computer simulations, they analyzed trends in interannual variation and found two powerful predictors of entomological risk of Lyme disease in a given year: abundance of tick hosts—white-footed mice and chipmunks—in the previous year and abundance of acorns—which sustain the rodents—two years out. Their findings upset the long-held view that deer and climate are the best indicators of disease risk.

I. scapularis larvae hatch in midsummer, and acquire infection after feeding on an infected mouse or other small animal. Larvae detach after several days of feeding, then molt into nymphs and enter a nearly year-long dormant stage. After another round of feeding, nymphs fall off and molt into adults, which prefer the blood of larger mammals. Larvae and nymphs can acquire and transmit infection, but people are most likely to contract Lyme disease from nymphs.

A person's risk of exposure to Lyme disease depends on the population

density of infected nymphal ticks, which is a product of the total density of nymphs and the nymphal infection prevalence. (Humans can reduce their personal risk by using repellents and routinely checking for ticks when visiting high-risk areas.) Many studies have examined variations in climate and white-tailed deer population dynamics as determinants of tick abundance and disease risk. But few have investigated the impacts of fluctuations in the abundance of hosts for larval ticks, and none have examined all of these variables—temperature, precipitation, deer, mice, chipmunks, and acorns—simultaneously over such a long period.

From 1991 to 2004, the researchers collected temperature and precipitation data, and estimated the abundance of acorns and animals on six plots of land. From this 13-year dataset, they developed computer models to estimate how each of the 11 variables (including multiple climate and deer indexes) contributed to yearly variations in the density of infected ticks and thus risk of human exposure.

While none of the climate variables influenced nymphal infection prevalence, higher temperatures in the previous year and precipitation patterns in the current year had weak, though unexpected, effects on total density and density of infected nymphs. It's thought that higher temperatures keep tick populations down, but the models showed them increasing both total density and density of infected nymphs. And though tick survival is expected to rise with precipitation, the models found the highest tick numbers at intermediate precipitation levels. These inconsistencies can be explored by incorporating other variables with documented effects into the approach outlined here. Also surprising, the researchers found that even a 3-fold variation in deer numbers had no impact on subsequent nymph abundance.

Density of infected nymphs—the principal determinant of Lyme disease risk—varied significantly from year to year, fueled mostly by large fluctuations in total nymph density, which in turn depended mostly on fluctuations in abundance of acorns, mice, and chipmunks. Interestingly, though chipmunk densities are generally lower than mice, their numbers were the best predictor of total nymph density in the subsequent year, likely reflecting their inferior grooming skills. Overall, the results found that acorns were the best predictor of Lyme disease risk—stemming from their crucial role in supporting white-footed mice, chipmunks, and likely other small animals, which in turn provide large reservoirs for *B. burgdorferi*. Acorns will not be a universal predictor of risk, the researchers acknowledge, since the disease occurs in areas without oaks. But the strength of these findings suggests that the observed link between increased Lyme disease risk and high rodent densities indicates that important food sources—or predators—of the rodent hosts of nymphs will be valuable predictors of disease risk.

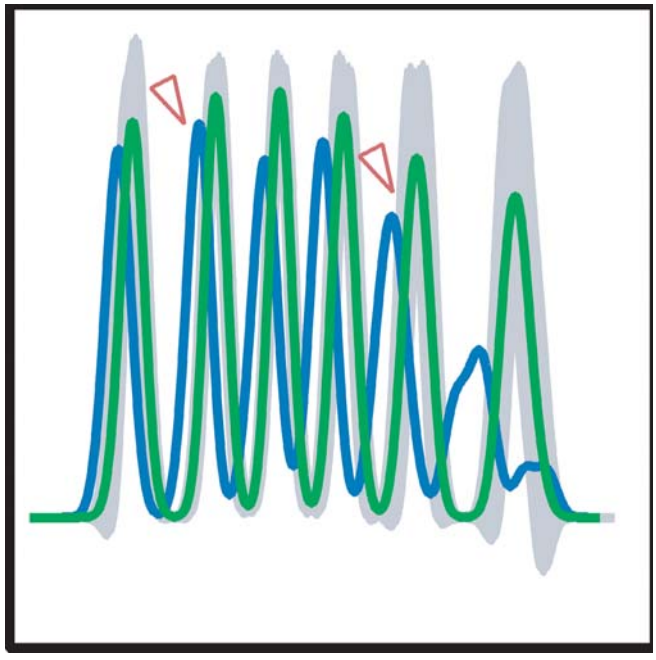
Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F (2006) Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. DOI: 10.1371/journal.pbio.0040145

Membrane Oscillations Keep Neurons on the Right Track

Liza Gross | DOI: 10.1371/journal.pbio.0040191

Whether you are asleep or awake, engaged in flights of fancy, or engrossed in intense concentration, your brain is a hotbed of electrical and chemical activity. Nerve cells have channels that regulate the flow of ions in and out of their membrane. In a resting neuron, the potential inside the cell is negative, averaging about −70 millivolts, compared with the outside. Signals generated by a sensory stimulus drive the membrane potential to a less negative, depolarized, threshold value (about −55 millivolts). The neuron then releases a stream of electrical activity—as series of action potentials, or spikes—along its membrane. After firing an action potential, the membrane potential becomes even more negative than when at rest (called hyperpolarization), and the neuron resists firing for a variable period of time. The timing and firing rate of such spikes are all the information the brain has to represent a given sensory stimulus.

All the while neurons should be conveying precise signals, they are exposed to various sources of electrical noise. This noise causes "jitter"—tiny fluctuations in the timing of any single action potential with respect to the signal that can accumulate and affect the accuracy of a transmitted message. On top of this noise, a neuron's resting potential oscillates around the resting value, well below the action potential threshold. Interestingly, and counterintuitively, increasing evidence suggests that this additional variability may help neurons separate signal from noise. In a new study, Troy Margrie and colleagues have used a combination of experimental and theoretical approaches to investigate the



DOI: 10.1371/journal.pbio.0040191.g001

A mean peri-stimulus time histogram for two stimuli (blue and green) in the presence of a background oscillation. The gray shading represents the variance of the green stimulus; oscillations permit the discrimination of stimulus-specific situations (red arrows).

role of membrane potential oscillations (MPOs) in signal processing. They show that oscillations in a single cell improve the precision of an action potential by reducing the accumulation of jitter that is inherent during ongoing spiking.

It has been suggested that spike sequences (called spike trains) in hippocampal and olfactory cells adjust their timing to the phase of the cells' MPOs, thereby providing action potentials with a frame of reference that enhances signal processing. MPOs in the olfactory system are tied to the breathing cycle. To artificially manipulate the electrical signals in individual neurons and determine how MPOs affect action potential precision, the researchers recorded intracellularly from individual neurons in double tracheotomized mice. Since the overall timing of action potentials was the same for tracheotomized as compared with free-breathing mice, this meant the researchers could use the tracheotomized animals to compare action potential precision under oscillatory and nonoscillatory (control) conditions. They found that oscillations "greatly enhanced" the overall precision of the action potentials—though precision decreases with the number of preceding action potentials within an oscillation cycle.

To further investigate the source of this precision, the researchers recorded from mitral cells in olfactory bulb slices and explored the mechanism of enhanced precision by introducing alternating pulses of depolarizing and hyperpolarizing currents to simulate the natural oscillation cycle. Jitter accumulated with long periods of depolarization, but when a hyperpolarizing current was delivered between two action potentials, the second spike recovered the precision of the first. Longer hyperpolarizing periods "dramatically reduced" the variance of the membrane potential—and yielded the most precise action potentials. These results support their in vivo evidence that spike trains within an oscillation cycle lose their enhanced precision since the trough of the oscillation is absent during such a spike train.

With enhanced precision comes enhanced performance. Exposing different types of neurons to over 77 stimulus pairs showed that MPOs significantly increased a neuron's ability to distinguish temporally distinct stimulus patterns. Using simulations to vary the frequency and amplitude of the oscillations and the kinetics of the stimulus currents, the researchers found enhanced stimulus discrimination over "physiologically realistic ranges" of firing rates, input kinetics, and oscillation parameters. For low oscillation frequencies, however, stimulus discrimination is most robust when synaptic inputs arrived at the trough and early rising phase of an oscillation cycle.

By establishing a hyperpolarizing period, oscillations prevent jitter accumulation, which improves action potential precision and stimulus discrimination. That this feature occurs in different types of neurons suggests a relatively simple and general underlying mechanism, the researchers argue. Any system where inputs must be integrated and transformed into discrete outputs, from circadian rhythms to gene expression programs, would likely benefit from any reset mechanism that minimizes the accumulation of noise.

Schaefer AT, Angelo K, Spors H, Margrie TW (2006) Neuronal oscillations enhance stimulus discrimination by ensuring action potential precision. DOI: 10.1371/journal.pbio.0040163

X Chromosomes' Shape-Shifting Foreshadows Random Inactivation

Françoise Chanut | DOI: 10.1371/journal.pbio.0040195

Molecular biology often seems like it leaves nothing to chance: signal A triggers reaction B, which activates enzyme C and silences gene D—leading cells to divide, differentiate, or die. But chance does sometimes meddle with molecular biology, as shown for instance by the apparently random inactivation (silencing) of the X chromosome. Indeed, in mammals, one copy of the X chromosome becomes silent early in the development of female embryos. As a result, female cells, which have two X chromosomes, produce the same amount of X-encoded molecules as male cells, which have only one. But while male cells have no choice as to which X to keep active, female cells do. That they leave that decision to chance is illustrated by the fact that female tissues are mosaics of cells with either the paternal or the maternal X chromosome in the silent state.

Precisely when chance comes into play during X inactivation is unclear. The prevailing theory is that the X chromosomes of female embryos are equivalent until the onset of inactivation, at which point the chromosomes' fates as active (Xa) or inactive (Xi) become sealed through chance selection. But Susanna Mlynarczyk-Evans, Barbara Panning, and colleagues challenge this theory with a new study that shows that X chromosomes oscillate between two states with different potentials for inactivation before they commit to the Xa or Xi fate. Their observations suggest that random X inactivation reflects a much earlier randomization of X chromosome states than previously thought.

DOI: 10.1371/journal.pbio.0040195.g001

Chromosome-wide differences prior to X inactivation may underlie the random choice of which X chromosome will be silenced.

Mlynarczyk-Evans, Panning, and colleagues studied mouse embryonic stem (ES) cells, which mimic in culture the early growing stages of embryonic development, before differentiation triggers X inactivation. Using fluorescent probes specific to various X-encoded genes, the researchers observed that in approximately half of female ES cells, a gene would appear as a single spot (S) on one X and a doublet (D) on the other. Moreover, nearby genes on the same chromosome

most often displayed the same S or D status. In any culture of actively dividing cells, one might expect a gene to appear as a singlet before DNA replication, and a doublet after replication. But using a variety of tests, the researchers demonstrated that S signals persisted after X chromosome replication. On the other hand, S signals were sensitive to experimental procedure, and split into two when cells underwent a staining protocol known to disrupt chromosome architecture. The researchers concluded that the X chromosomes of proliferating, undifferentiated ES cells exist in two different physical states.

Curious about what these different states might portend, the researchers repeated their experiments in ES cells carrying various mutations that bias X inactivation. For instance, in cells that harbor a wild-type X and an X mutant for the *Xist* gene, the wild-type X always becomes the Xi. In a large proportion of such cells, the researchers saw that the wild-type X had mostly D signals, and the mutant mostly S signals. Similar biases were observed with other mutations, suggesting that S and D signals (and the different physical states they reveal) might be early markers of the Xi and Xa fate.

But if wild-type X chromosomes are already predisposed for a future Xi or Xa fate before inactivation begins, how is randomness achieved? A possible answer to that question is that pre-inactivation states are unstable. To address this possibility, the researchers established cultures from single ES cells that harbored two wild-type chromosomes, one of which was easily distinguished from the other because it had fused to Chromosome 2. Each founder cell presumably carried either the normal or the fused X in the pre-Xi or pre-Xa state. Yet, in the progeny of each founder, both chromosomes were found in either state in equal proportions. Hence, X chromosomes seem able to switch from one state to the other during cell divisions, quickly achieving a random distribution of the pre-Xi and pre-Xa states.

Mlynarczyk-Evans, Panning, and colleagues do not yet understand the biochemical mechanisms that allow X chromosomes to switch states coordinately in dividing ES cells. But their observations suggest that the differentiation signal that triggers X inactivation might not initiate randomness, but simply stabilize the state of already randomized, shape-shifting chromosomes.

Mlynarczyk-Evans S, Royce-Tolland M, Alexander MK, Andersen AA, Kalantry S, et al. (2006) X chromosomes alternate between two states prior to random X-inactivation. DOI: 10.1371/journal.pbio.0040159

Polycombing *Drosophila* for Dynamic Developmental Footprints

Emma Hill | DOI: 10.1371/journal.pbio.0040177

In some ways, the developmental passage of a fruit fly from a ball of cells through discrete morphological stages—embryo, pupa, and larva—to a fully formed organism really showcases the potential of biology. Organism development (in this case of a fly) requires a temporally synchronized regulation of a myriad of genes and pathways across the *Drosophila* genome.

No single component of these developmental processes can be considered more important than another—a mistake anywhere can prove fatal. One set of participants in *Drosophila* development are the Polycomb group (PcG) proteins, which bind to regions of chromatin (the genomic DNA twisted around histone proteins that helps structure the chromosomes) and silence the transcription of nearby genes. A handful of PcG response elements (PREs—the regions of the genome where PcGs bind) have been characterized to date, but until now not much was known about their broader distribution across the genome. Nicolas Nègre, Giacomo Cavalli, and colleagues provide just such a map of PcG binding locations across chromosomes X and 2 of *Drosophila melanogaster*. They observe a wide distribution that varies from one developmental stage to the next.

PcG proteins actually form multiprotein complexes, one of which—the PRC1 complex—contains two specific proteins, PC and PH. PC and PH have in the past been seen to co-localize with another protein called the GAGA-factor, or GAF. Nègre and colleagues mapped the binding locations of individual PC, PH, and GAF proteins (separately) to DNA from embryos, pupae, and adult male and female flies. They did this with a technique called "ChIP on chip," which identifies matches between the region of chromatin where the protein of interest has bound (via chromatin immunoprecipitation, or ChIP) and the corresponding genomic region on a tiled microarray (or chip).

DOI: 10.1371/journal.pbio.0040177.g001

Mapping the chromosomal distribution of Polycomb group proteins in *Drosophila* to study the developmental maintenance of cell identity.

To double-check that their matches were sensible, some known PREs were placed on the microarrays (acting as positive controls), along with some randomly selected DNA fragments unrelated to any known PREs (negative controls). To confirm that the chromosomal locations of

the ChIP-on-chip matches corresponded with the correct region of the *Drosophila* genome, the authors also undertook some fluorescence mapping experiments and, reassuringly, observed perfect co-localization of sites mapped by ChIP on chip with sites mapped on the chromosomes by cytology (observing the band on the chromosome that fluoresces).

So how do these proteins distribute over the genome? As expected, PC and PH showed almost identical distribution, which confirms their presence together in the PRC1 complex. GAF binding did not overlap perfectly with the PRC1 proteins and actually bound to more sites in total. GAF also tended to bind to narrower chromatin regions, while PC and PH were more often spread over larger regions. These results indicate that PC and PH binding interactions do not depend on GAF.

Interestingly, the PRC1 proteins frequently bound in clusters, and so it seems that in some genomic regions, more than one PRE must exist in proximate locations. The genes nearest to the PREs, as expected, are most frequently biased toward regulatory and developmental functions. Their distances from the PREs, however, are variable, and it is likely that the PcGs must use a variety of mechanisms to turn off transcription of these nearby genes (perhaps by binding on top of the genes' promoter region, or maybe by linking multiple PREs to interfere with more distant promoters).

How did the binding of these proteins differ across the different developmental time points? While the positions of GAF localization remained quite constant, the PcG protein binding sites did not. Although some PREs were maintained at all times, other PcG protein locations varied considerably, implying that PcGs employ dynamic regulation over time. In fact, adult male flies had the most divergent profile of PREs, suggesting that some male-specific development might be regulated by these PcG proteins.

Nègre, Cavalli, and colleagues show that these PcG proteins do indeed constitute a critical component of *Drosophila* development and are likely to instruct a variety of developmental processes through their regulation of genes across the genome. The next exciting steps in better understanding the molecular construction of a fly will be to extend this analysis to the remaining *Drosophila* chromosomes, across other developmental stages, and in different tissue types. From there, scientists can similarly probe what makes a mammal, and the extent to which these mechanisms are conserved across distant species.

Nègre N, Hennetin J, Sun LV, Lavrov S, Bellis M, et al. (2006) Chromosomal distribution of PcG proteins during *Drosophila* development. DOI: 10.1371/journal.pbio.0040170

The More the Merrier: Multiple Genomes in a Parasitic Prokaryote

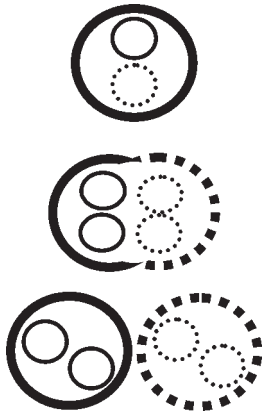
Liza Gross | DOI: 10.1371/journal.pbio.0040212

The notion that bacteria and other single-celled "prokaryotes" carry only one genome copy per cell is as ingrained as the notion that they lack a distinct nucleus. (Organisms with one genome copy are "haploid"; organisms with two, including humans, are "diploid"; and organisms with more than two, such as plants, are "polyploid.") This assumption is based on decades of research on *Escherichia coli* and a select group of other bacterial species.

But researchers have been finding indications that some bacteria, under certain conditions, have more genetic material in a single cell than would be expected for a single genome. And now, using a combination of cell sorting, fluorescent microscopy, microarray, and quantitative PCR techniques, Deborah Tobiason and Hank Seifert present strong evidence that the bacterium that causes gonorrhea, *(Neisseria gonorrhoeae)*, is indeed polyploid.

N. gonorrhoeae successfully infects at-risk individuals by reshuffling the genetic code for proteins expressed on its hairlike "pili" protrusions through a process called gene conversion. During gene conversion, one gene incorporates DNA from a variant copy that carries slightly different information. The high frequency of gene conversion events enhances pili protein diversity, which increases the likelihood that the human immune system won't detect the pathogen. It is conceivable that *N. gonorrhoeae* might briefly express two copies of the pilin gene in one cell after DNA replication, before cell division. But Tobiason and Seifert thought that the high frequency of gene conversion required for antigenic diversity might instead be supported by the presence of extra genome copies.

To explore this possibility, the researchers had to distinguish the amount of DNA associated with genome duplication prior to cell division from that found in a nondividing cell. They labeled the DNA of *N. gonorrhoeae* with fluorescent markers and, as a source of comparison, did the same for *E. coli*, which has provided a wellspring of knowledge on chromosomal DNA replication. Cells were either left untreated, allowing exponential growth, or were treated with an antibiotic that inhibits new rounds of replication but allows any DNA replication already in progress to finish. Cells in "stationary phase"—during which cell division stops and no new DNA replication begins, but active replication forks complete their task—were also labeled. Both antibiotic treated cells and stationary cells produce fully replicated chromosomes.



DOI: 10.1371/journal.pbio.0040212.g001

The obligate human pathogen, *Neisseria gonorrhoeae*, has multiple genome copies per cell, which may be necessary for antigenic diversity and survival within the human host.

Using a standard cell sorting technique called flow cytometry, the researchers separated cells based on their DNA content. Treated and stationary phase *E. coli* cells produced

individual cells with the equivalent of two, four, or eight genomes per cell. Since multiple initiation events on the chromosome can occur per round of cell division, the number of replicated genomes reflects the number of replication forks active when cell division stops. Untreated *E. coli* cells, however, revealed a wide range of DNA content per cell, likely reflecting the presence of multiple replication forks at various stages of replication and cell division. The DNA content of untreated gonococcal cells also varied, with most cells having between four and six genome equivalents.

Gonococcus exists as single (monococcal) and double spheres (diplococcal)—it is these two forms, Tobiason and Seifert show with fluorescence microscopy, that explains the range of DNA content in different cell populations found with flow cytometry. Monococci had, on average, about three to six genome equivalents, while diplococci averaged about four to ten per cell. To explore the reason for the expanded DNA content, the researchers treated gonococcal cells with antibiotics to generate fully replicated chromosomes. These experiments suggested that actively dividing cells have two or four pairs of active replication forks. They used independent measures to confirm that both monococci and diplococci contain fully replicated chromosomes with a single pair of active replication forks on each chromosome—which means that the DNA content they saw in gonococcal cells is not the result of multiple replication initiation events on a single chromosome, as occurs with *E. coli*, but represents multiple, fully replicated chromosomes. Future studies will have to determine whether polyploidy underlies pilin antigenic variation and the bacterium's success as a human pathogen. But this study leaves little doubt that it's time to retire the notion that all prokaryotes are haploid.

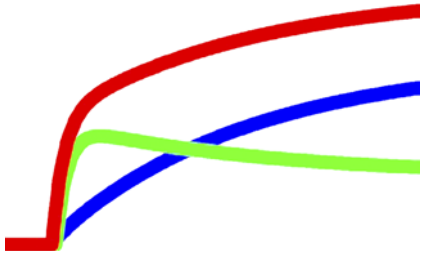
Tobiason DM, Seifert HS (2006) The obligate human pathogen, *Neisseria gonorrhoeae*, is polyploid. DOI: 10.1371/journal.pbio.0040185

A New Model of Short-Term Motor Adaptation

Liza Gross | DOI: 10.1371/journal.pbio.0040205

Starting at around three months old, children can finally reach for the countless toys their parents have been dangling before them since birth. These attempts often involve a good deal of flailing about, as motor skills, like anything else, require cultivation. Motor control depends on executing the proper musculoskeletal force to reach the desired object.

Prior learning facilitates motor control (all that flailing serves a purpose), which is aided by a fundamental feature of memory, called savings. When a novel response to a stimulus is learned in one set of trials, then "washed out" in an unlearning phase, subsequent relearning proceeds faster. Neuroscientists have been puzzled by savings and other features such as interference and rapid unlearning reported in adaptation studies because standard models of short-term motor adaptation couldn't explain them. But now Maurice Smith, Ali Ghazizadeh, and Reza Shadmehr have solved this puzzle by combining experimental and computational approaches, and show that two adaptive brain components with different learning rates and retention capacities can account for these seemingly disparate properties of learning.



DOI: 10.1371/journal.pbio.0040205.g001

Two learning processes with distinct time courses contribute significantly to short-term motor skill acquisition, and the interactions between these processes explain a host of puzzling phenomena in motor adaptation.

In a recent study of savings in eye-saccade adaptation (saccades are rapid eye movements that shift gaze direction) in monkeys, Yoshiko Kojima et al. explained the savings they observed with a model in which two distinct mechanisms increased or decreased the amplitude gain of saccades based on a target's direction. This "two-state, gain-specific" model explained the savings and washout effects the researchers had seen. But Kojima et al. found that when adaptation was extinguished, and then time passed with the monkey receiving no feedback on its behavior, there was a sudden recovery of the animal's saccade gains toward the initial learning state. This spontaneous recovery could not be explained by the gain-specific model. Nor, Smith et al. explain, can the gain-specific model explain a property of learning called anterograde interference, in which initial motor learning is faster than subsequent adaptation to an opposing task.

Smith et al. focused on the spontaneous recovery phenomenon: since behavior changed even when there was no error feedback as a guide, they reasoned that motor error could not be the only factor affecting motor learning and that passage of time must have also played a role. They considered a system in which two states both learned from error: one learned rapidly but tended to quickly forget, while the other learned slowly and tended to remember.

To test their hypothesis, they first designed a variation on a standard paradigm for studying how humans learn to compensate for an imposed force while reaching for a target. Participants held a joystick-like, robot-controlled "manipulandum," which measures hand position and velocity, and applies well-controlled counter forces to the hand. Participants' hand position was visible on a computer monitor in front of them, and they were told to quickly reach toward small circular targets spaced ten centimeters apart along a horizontal plane. After practicing without the robot motors, participants adapted their reaching movements to a force-field perturbation pushing their hand in one direction; then learning was extinguished by adaptation to an opposing force field. Error feedback was then removed with an "error clamp" that forces the hand along a straight line to eliminate lateral errors during movement. Even though participants were kept from experiencing lateral errors, the manipulandum recorded their

lateral force resistance to the error clamp, so the researchers could infer participants' motor plans and track the evolution of learned changes in motor output.

As predicted by the model, the observed spontaneous recovery suggested that two different learning states—one with a fast time scale and another with a slower time scale—combined to produce motor output. Rebound occurs because the fast-learning module decays quickly during the no-feedback block, but the slow-learning module decays gradually, allowing a transient recovery of motor output.

Altogether, these results argue for a learning system based on two time courses and explain a wide range of phenomena associated with short-term motor adaptation. The multirate model accounts for anterograde interference, by showing that the slow learning component is initially biased against learning the second adaptation. And it explains "rapid unlearning"—in which the rate of de-adaptation after a period of learning can be much faster than initial learning—and additionally predicts that this de-adaptation is fastest after short adaptation blocks, then declines as the amount of training in the initial adaptation increases.

It may well be that different functional units in the brain learn motor control at different rates—a possibility that researchers can explore with the help of this unifying model of motor adaptation. And by combining behavioral experiments with targeted brain lesions and functional imaging in the cerebellum—which is required for normal motor adaptation—researchers can investigate what regions of the brain control these learning modules.

Smith MA, Ghazizadeh A, Shadmehr R (2006) Interacting adaptive processes with different timescales underlie short-term motor learning. DOI: 10.1371/journal.pbio.0040179

Mating Patterns and Bt Resistance: The Case of the European Corn Borer

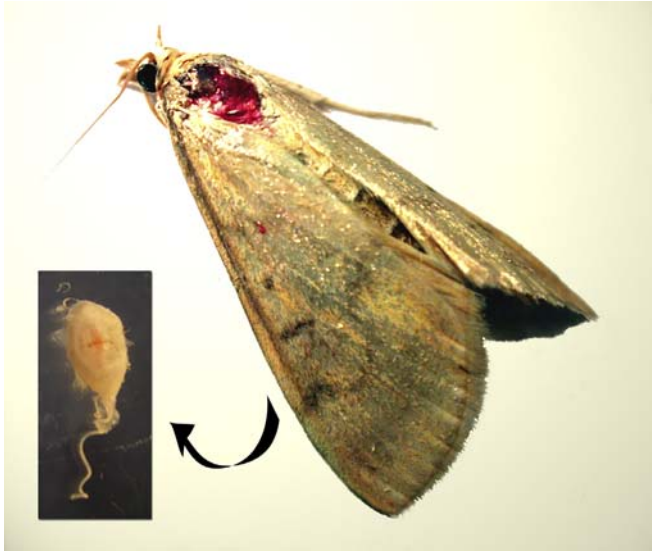
Liza Gross | DOI: 10.1371/journal.pbio.0040214

The main argument for planting transgenic, pest-resistant crops rests on their potential to reduce widespread pesticide spraying. Such benefits require that agricultural land-use strategies impede the evolution of pest resistance. But such strategies—including the high-dose refuge (HDR) strategy mandated in the United States—are based on key assumptions about pest biology. Insect fitness depends on finding the optimal balance between when to mate and when to set off for greener pastures—mating before dispersal increases the risk of inbreeding, but dispersing before mating increases the risk of never finding a mate. Pest management depends on understanding and using this balance to prevent the evolution of insect resistance.

As important as mating and dispersal are to thwarting resistance, empirical evidence on these behaviors in major crop pests—including the European corn borer *(Ostrinia nubilalis)*, targeted by *Bacillus thuriengiensis (Bt)* toxins produced by transgenic insecticidal corn—is limited. While European corn borers *can* disperse over half a mile—the maximum distance allowed between a *Bt* field and the corresponding refuge—and even up to 20 miles, it's unclear whether they do so routinely, take flight before or after mating, or show any preference for resident or immigrant mates.

To gain a better understanding of the European corn borer's mating and dispersal habits, Ambroise Dalecky, Sergine Ponsard, Richard Bailey, Céline Pélissier, and Denis Bourguet released and recaptured moths in French crop fields over three breeding seasons in 2004 and 2005. They found that females mate randomly with resident and immigrant males—an important HDR assumption, since random mating dilutes resistance genes. But they also discovered that some males and females mate before dispersing—suggesting a possibly more restricted mating range than previously assumed.

The HDR strategy assumes that a patchwork of *Bt* cornfields interspersed with untreated refuges will facilitate gene flow between the areas. Resistance gene variants, or alleles, are found in either one (heterozygotes) or two (homozygotes) copies in individuals who carry them. Only homozygotes are resistant to (that is, survive the *Bt* toxins), while heterozygotes



DOI: 10.1371/journal.pbio.0040214.g001

Dispersal and mating patterns in European corn borer moths: a color-marked female and a biogeochemically marked spermatophore (shown at about the same scale). (Image: Dalecky et al., Laurent Pélozuelo, and Laurent Soldati)

die as larvae if they feed on such plants. HDR management aims to eliminate as many resistance alleles from each generation as possible by maximizing the proportion of heterozygotes (where resistance alleles are available for purging) and maximizing the proportion of these heterozygotes developing in *Bt* fields (where resistance alleles are indeed purged), rather than in refuges. If substantial mating takes place before dispersal, there will be more homozygotes, which means fewer purging opportunities—and a potentially less effective HDR strategy. If crops are not rotated and if post-mating dispersal is low, this deleterious effect of low pre-mating dispersal can be offset by having a higher proportion of heterozygous eggs laid on *Bt* corn plants and dying at larval stage. In other cases, however, low

pre-mating dispersal is predicted to reduce the efficacy of the HDR strategy.

To track moth mating and dispersal habits, the researchers relied on biochemical markers—consisting of different carbon signatures produced by wheat and corn, which are in turn evident in lab-raised, wheat-fed moths, and corn-fed field moths—and color-coded ink spots applied to adults before release. Male and female pupae were separated to prevent mating before they were released less than 24 hours after they emerged from their pupal case.

In 17 sessions over three weeks, nearly 8,800 virgin moths were released during the June 2004 breeding season into four different cornfields. Soybean and wheat—crops that aren't targeted by corn borers—grew in the fields the year before, so no pupae were present as if the field had been planted with *Bt* corn. Refuge facsimiles were provided by adjacent fields planted with corn in 2003, the most likely source of wild moths at the 2004 study sites.

Thirty-six hours after release, marked moths and unmarked moths were captured along the field borders, where the moths typically mate and rest. The proportion of marked individuals that were recaptured varied significantly between sites and sessions but not between sexes, averaging about 4%. About 97% of recaptured females had mated—indiscriminately with resident and immigrant males, the researchers determined, by analyzing the carbon content of the sperm packets, or spermatophores, they acquired during mating. Resident males rarely mated with immigrant females, perhaps because immigrant females tended to arrive already mated.

Because wild moths might disperse sooner than 24 hours, the researchers placed corn borer pupae directly in a cornfield the following season, then watched and waited. Moths first took wing about 3.5 to 9.5 hours after emerging, and their flight movements were unlikely to take them farther than 220 feet within the first 24 hours—indicating that high rates and high distances of dispersal were unlikely to occur before mating and that far more than 4% were still present and hence likely to mate within their natal range.

The researchers go on to confirm that a "substantial proportion"—up to about 57% for females—of newly emerged corn borers mate at a very local scale before dispersing. These findings suggest that the HDR strategy may not ensure substantial gene flow between susceptible and resistant moths. This disconnect between the evidence and the HDR model indicates that other assumptions—including whether females who mate close to home also lay their eggs there—should be rigorously tested before counting on HDR to circumvent the resistance machinery of its targets.

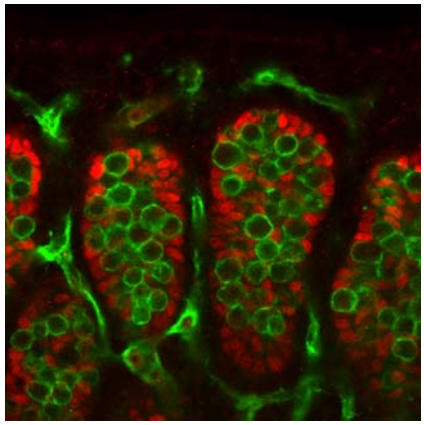
Dalecky A, Ponsard S, Bailey RI, Pélissier C, Bourguet D (2006) Resistance evolution to *Bt* crops: Predispersal mating of European corn borers. DOI: 10.1371/journal.pbio.0040181

Male or Female? It Depends on the Dose

Liza Gross | DOI: 10.1371/journal.pbio.0040211

In most cases, a fertilized egg with two X chromosomes will become a girl and an XY egg will become a boy. Molecular analysis of the genomes of sex-reversed individuals identified the sex-determining region Y gene *(Sry)*, the primary sex-determining gene in mammals. The presence of an *Sry* gene normally drives male development. But when downstream elements of the sex-determining pathway are disrupted, chromosomal sex and sexual phenotype can be discordant. This phenomenon revealed a fatal flaw in a now-defunct Olympics rule requiring female athletes to submit to "gender verification" procedures that labeled XY females "imposters."

Every embryonic gonad—at this point, neither ovary nor testis—harbors cells with the potential to choose either fate (male or female). Reflecting this noncommittal approach, both XX and XY gonads initially display similar expression patterns for the sex-determining genes *Sox9*, *Fgf9*, and *Wnt4*. If *Sry* does not intervene during a critical window in development, the cells will default to the ovarian pathway. Once *Sry* expression begins, other genes start to choose sides: *Sox9* and *Fgf9* become active only in male gonads, while *Wnt4* becomes active only in female gonads. Loss of *Fgf9* in XY mice leads to sex reversal; in XX mice, loss of *Wnt4* leads to partial testis development. Only when the protein products of *Sry*, *Sox9*, and *Fgf9* are all expressed together do male-specific Sertoli cells develop—and prod the developing gonad toward full male differentiation.



DOI: 10.1371/journal.pbio.0040211.g001

Fgf9 is critical to repress Wnt4 and establish the testis pathway during mammalian sex determination. Sertoli cells (red), germ cells, and endothelial cells (green).

Clearly, *Fgf9* and *Wnt4* must somehow influence sex determination—but how? In a new study, Yuna Kim, Blanche Capel, and colleagues explored how *Sry*, *Sox9*, *Fgf9*, and *Wnt4* contribute to the initial events of sex determination in mice. By manipulating the activity of these signals, they show that *Wnt4* and *Fgf9* play a sexual tug of war. If *Wnt4* wins, the gonad differentiates into an ovary. If *Fgf9* wins, it turns into a testis. Living up to its name, *Sry* tips the balance in favor of *Fgf9*, by triggering expression of *Sox9*, and a male fate.

The researchers started by examining *Fgf9* expression during normal gonad development in the mouse. 11.5 days after conception, *Fgf9* was evenly distributed in the gonads of both sexes. By day 12, it was found only in XY gonads, and only in Sertoli cells within testis cords and cells near the surface of the gonad.

Seeing *Fgf9* expressed so early in testis differentiation suggested it might trigger *Sry* activity or expression, but the researchers found that this wasn't the case. If *Fgf9* doesn't regulate *Sry*, they reasoned, maybe it affects the male-specific up-regulation of *Sox9*, which is absent in XY mice lacking *Fgf9*. Adding

Fgf9 to XX cells isolated from 11.5-day-old gonads triggered increased *Sox9* expression. These findings showed that artificial expression of *Fgf9* can induce *Sox9* expression in single XX cells, but what about in the intact gonad?

In normal and mutant *Fgf9* XY mice, *Sox9* expression followed similar patterns for the first 24 hours after *Sry* was expressed. By 12.5 days, SOX9 proteins were no longer expressed in the mutant mice, and the cells that would normally have developed into Sertoli cells failed to organize into normal testis cords. These results, the researchers conclude, indicate that *Fgf9* isn't necessary to trigger *Sox9* expression, but instead is required to maintain *Sox9* expression in Sertoli precursor cells.

Fgf9 expression depends on *Sox9*, and *Fgf9* returns the favor by maintaining *Sox9* expression in a positive feed-forward loop. The feed-forward loop between these genes allows the proliferation and expansion of Sertoli and other somatic cells in XY gonads. Without *Fgf9*, XY gonads showed a rapid drop in SOX9 protein levels and expressed few or no Sertoli cell markers. *Wnt4*, on the other hand, increased, suggesting it was repressed by *Fgf9*. Without the antagonistic effects of *Fgf9*, *Wnt4* predominates, disrupting the *Fgf9–Sox9* pathway and establishing the infrastructure necessary to become female. And the reverse holds true: reducing *Wnt4* levels in XX gonads allows FGF9 and SOX9 levels to increase—even in the absence of *Sry*—though other male factors appear to be required to establish testis development.

Altogether, these results suggest that sex is not determined by the flip of a genetic switch but by a dose-dependent interplay between opposing signals. The researchers propose that *Sry* initiates the male fate by triggering *Sox9* expression, which activates *Fgf9*, and sets off a feed-forward loop that produces a male, barring the interference of antagonistic signals. Whether these signals work within or between cells is not clear. It may be, for example, that *Fgf9* recruits other cells to the Sertoli pathway by triggering increased *Fgf9–Sox9* expression, or that *Fgf9* causes Sertoli cells to proliferate until they reach the critical mass needed to fend off *Wnt4* and secure the male pathway. Future studies can investigate the molecular mechanisms of *Fgf9* and *Wnt4*—and whether these competing pathways regulate the battle of the sexes in a wide range of vertebrates.

Kim Y, Kobayashi A, Sekido R, DiNapoli L, Brennan J, et al. (2006) *Fgf9* and *Wnt4* act as antagonistic signals to regulate mammalian sex determination. DOI: 10.1371/journal.pbio.0040187

Trapped: A Molecular-Level Look at How Herpes Disables Its Immune Destroyer

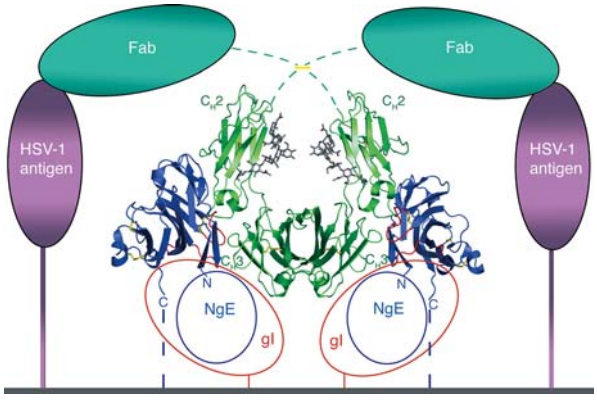
Mary Hoff | DOI: 10.1371/journal.pbio.0040189

It's not easy coming out ahead in the virus–host battle: the mechanisms living things have for seeking and destroying invaders, as elaborate as they might be, often find themselves up against equally elaborate viral mechanisms for evading them.

Such is the case when it comes to humans versus herpes simplex virus type-1 (HSV-1), a highly contagious virus that is virtually impossible to get rid of once it becomes established. When our bodies detect HSV-1 inside us, they mass produce antibodies called immunoglobulin G (IgG). These Y-shaped defense molecules latch onto antigens on the surface of the virus and virus-infected cells with their two arms, disabling them and marking them for pickup by the immune-system equivalent of roving trash trucks. But HSV-1 has a cagey way of turning the tables. A protein pair that it stations on its surface (and on the surface of cells it has taken over) known as gE-gI can grab IgG by its tail (known formally as its Fc region), incapacitating it instead.

Elizabeth Sprague, Pamela Bjorkman, and colleagues explored this interaction by focusing their attention on CgE, a portion of gE. Using an assay that measures how well two substances stick to each other, they showed that CgE binds Fc even in the absence of gI or other gE domains. They then used a technique that compares how crystals of CgE diffract X rays of various wavelengths to determine the three-dimensional structure of CgE.

Next the researchers attempted to create a three-dimensional picture of the gE-gI/Fc complex. Using the new information about CgE's structure, low-resolution images of gE-gI/Fc crystals, and techniques that make it possible to infer complete structure from partial information, they proposed a structure for the gE-gI/Fc complex in which two CgE components of gE-gI hold Fc like two hands holding a basketball. To substantiate that surmised structure, they applied a computational approach called protein docking, using what they knew about the structure of unbound CgE and Fc to calculate what complex would be energetically most favorable. Of the five plausible models they came up with, one was remarkably similar to the structure derived through the previous process, confirming its likelihood as the correct structure.



DOI: 10.1371/journal.pbio.0040189.g001

The three-dimensional structure of a gE-gI/Fc complex suggests that an anti-HSV IgG bound by gE-gI is in an upright orientation, positioning it to also bind its antigen on the surface of the same cell.

The researchers then used the newfound structural information to further explore the gE-gI/Fc interaction. They compared CgE with other proteins and a peptide of known structure that bind with the same region of Fc. They also used the structural information to explain on a molecular level the previously known inability of an Fc

mutant, another human immunoglobulin, and rodent IgG to bind with gE-gI.

One fascinating mystery the structures helped the researchers address is the fact that gE-gI binds well with Fc at neutral or slightly basic pH, but not in an acidic environment. The researchers showed that CgE-Fc binding is pH dependent, and attributed this trait to four histidine amino acids at the CgE/Fc interface that would likely alter the complex's chemistry in the presence of spare protons. They also speculated that the pH sensitivity was part of a viral strategy for attacking IgG in which gE-gI/IgG is drawn into the cell where the acidity causes the antibody to dissociate and eventually to be destroyed.

The structural studies also shed light on previous findings regarding the effects of mutations in gE on its ability to bind IgG and on cell-to-cell spread of HSV-1. Using their knowledge of CgE and gE-gI/Fc structures, the researchers were able to identify the structural and functional implications of various mutations and to pinpoint specific regions of CgE implicated in cell-to-cell spread.

Finally, the researchers used the structures to determine that gE-gI/IgG complexes likely orient upright in the cell membrane, leaving the arms of the gE-gI-bound IgG available to attach to a nearby HSV-1 antigen—a previously hypothesized state known as bipolar bridging. If bipolar bridging does indeed occur, subsequent endocytosis would then swallow up not only IgG but also any antigens involved in bridging, further hindering efforts to win the battle against this tricky viral invader.

Sprague ER, Wang C, Baker D, Bjorkman PJ (2006) Crystal structure of the HSV-1 Fc receptor bound to Fc reveals a mechanism for antibody bipolar bridging. DOI: 10.1371/journal.pbio.0040148

A Novel Phage Protein Mediates the Virus's Removal from Bacterial Chromosomes

Liza Gross | DOI: 10.1371/journal.pbio.0040213

Long before anyone realistically entertained the notion of engineering transgenic corn, cloned dogs, or designer babies, scientists looked for clues to the fundamental properties of life in much simpler organisms. Studies in one such organism—bacteria-infecting viruses called bacteriophages, or phages—produced some of the most important discoveries of molecular biology: identifying DNA as the genetic material, elucidating the mechanism of genome replication, and revealing the power of recombinant DNA, the backbone of genetic engineering.

Nearly 60 years after phage's golden era, scientists are still using the virus to illuminate the inner workings of the cell. And now in a new study, Pallavi Ghosh, Laura Wasil, and Graham Hatfull describe a novel phage protein that uses an unusual mechanism to induce the infectious stage of the phage life cycle.

Lacking the means of self-replication, phages inject their genome into a bacterial cell and co-opt its molecular machinery to mass produce new viruses, ultimately destroying the cell to release its progeny. Temperate phages can reproduce through this lytic life cycle or enter a lysogenic life cycle during which they integrate their DNA into the bacteria's genome as a noninfectious "prophage." The prophage remains harmless until it is excised from the bacterial genome.

Both integration and excision depend on the combined efforts of integrases and recombination directionality factors (RDFs) in a

DOI: 10.1371/journal.pbio.0040213.g001

The mycobacteriophage Bxb1 uses a novel enzyme to remove itself from its bacterial host's genome before launching an infection.

cut-and-connect operation called site-specific recombination. During integration, integrases mediate DNA-strand exchange between phage DNA—at the phage attachment site, *attP*—and bacterial DNA—at the bacterial attachment site, *attB*. Successful integration creates junction sites—*attL* and *attR*—on both sides of the integrated prophage that mark future excision sites. RDFs can catalyze integration or excision, typically by binding to DNA near the respective recombination sites and forming a complex with integrase that remodels the DNA and creates macromolecular structures that promote one process while inhibiting the other.

While much is understood about how tyrosine integrases (so-called because the amino acid tyrosine mediates the catalytic reaction) effect site-specific recombination, Ghosh et al. were interested in the more cryptic serine integrases. Serine integrases lack many of the features other enzymes use to regulate site-specific recombination, such as DNA-binding cofactors or DNA remodeling, and little is known about the few RDFs that have been linked to them. To learn more about serine integrase activity, the researchers worked with a phage called Bxb1 that uses a serine integrase to insinuate itself into the genome of the bacterium *Mycobacterium smegmatis*.

They first analyzed the Bxb1 genome to identify candidate RDFs using standard bioinformatic methods, but RDFs are too diverse to allow accurate sequence comparisons. So they turned to an experimental method to isolate the RDF, using a phage strain with genes organized akin to an integrated prophage, complete with recombination junctions *(attL* and *attR)* and integrase *(int)*. From this strain, they engineered an "excision tester strain" with one stretch of sequence conferring antibiotic

resistance and another conferring sucrose sensitivity (thus, stunting growth) inserted between the *attL* and *attR* junctions. Successful excisive recombination would remove the intervening sequence and confer sucrose resistance, allowing the strain to grow in sucrose.

To identify excision-related sequences, Ghosh et al. created a Bxb1 DNA library and incorporated it into the tester strain, producing colonies of genetically transformed strains. The resulting colonies produced sucrose-resistant strains, which had undergone site-specific excision recombination between *attL* and *attR*, the researchers confirmed, which had been mediated by a fragment of Bxb1 DNA and a putative RDF. And by creating genetic variants of the tester strains, they showed that excisive recombination requires both the Bxb1 integrase *gpInt* and a candidate Bxb1 RDF.

Only one region in this putative RDF, gp47, had been previously described. And though it is much larger and unlike any other known RDF, the researchers show that gp47 does indeed catalyze excisive recombination and can inhibit integration, like other RDFs. Bxb1 gp47 distinguishes itself, however, by regulating site-specific recombination through novel interactive mechanisms that don't require complex macromolecular structures or direct DNA binding. Instead, the researchers propose, it instigates excisive recombination through transient interactions with gpInt–*attL/R* junctions—and blocks integrative recombination through more stable interactions that prevent gpInt–*attB* and gtInt–*attP* complexes from forming productive associations.

Altogether, these results point to a novel RDF with an unusual strategy for regulating integration and excision. Though the gp47 RDF acts through protein–protein interactions rather than through direct DNA-binding interactions, its effects depend on the specific DNA site that gpInt binds. With evidence that gp47 also plays a role in DNA replication, the researchers plan to investigate whether this novel enzyme uses the same strategy to interact with other phage proteins—and maybe even to commandeer host machinery and unleash infection.

Ghosh P, Wasil LR, Hatfull GF (2006) Control of phage Bxb1 excision by a novel recombination directionality factor. DOI: 10.1371/journal.pbio.0040186

RNA Polymerase Promotes Splicing, Prevents Degradation

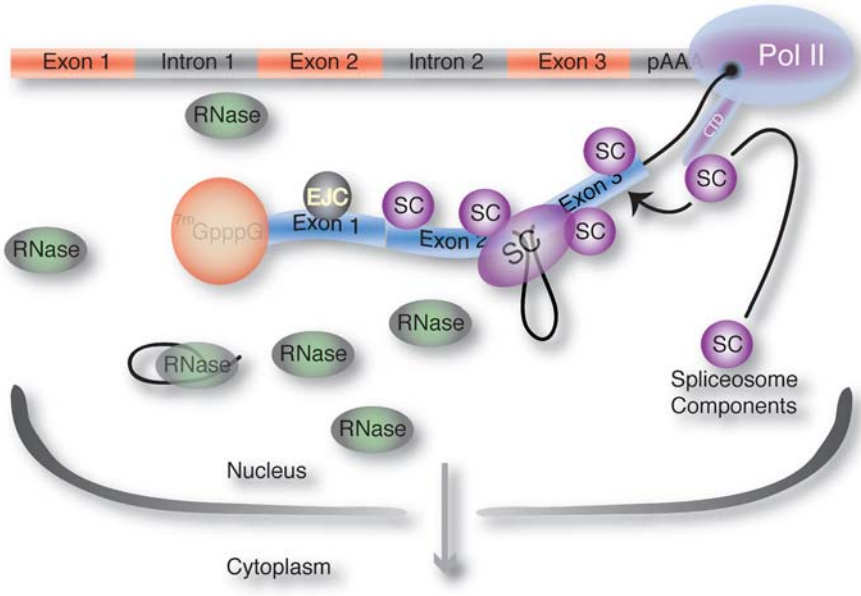
Richard Robinson | DOI: 10.1371/journal.pbio.0040183

As it rolls off the DNA assembly line, RNA faces an uncertain fate. Down one path lies further processing and, ultimately, translation into protein. Down the other, though, lies oblivion—degradation by RNA-digesting enzymes, and recycling back into the nucleotides from which it came. A new study by Martin Hicks, Klemens Hertel, and colleagues shows that efficient processing is promoted, and oblivion avoided, by a close linkage between the machinery of RNA synthesis and that of RNA processing.

Making a protein from a DNA gene requires first making an RNA copy, a process handled by the enzyme complex RNA polymerase II (pol II). This product, called pre-messenger RNA (pre-mRNA) gets a cap and tail, which protect it from exonucleases, enzymes that could otherwise degrade the RNA starting from its ends. They do not protect it from endonucleases, however, which can attack the middle of the RNA strand and render it useless. Endonucleases are prevented from attacking the pre-mRNA when it is bound by the splicing machinery, another enzyme complex that removes noncoding bits, called introns, and splices together the remaining exons.

To investigate whether pol II helps promote binding of the splicing machinery and thus prevent pre-mRNA destruction, the authors compared pol II, found in all animal cells, with a viral RNA polymerase, T7, in otherwise identical cell-free systems. Both generated similar amounts of pre-mRNAs, but spliced mRNAs were created much more efficiently in the pol II system. This might be due either to faster binding of the splicing machinery to the pre-mRNA or to faster processing of the RNA once it is bound. By analyzing the reaction mixture at different time points, the authors found that binding, not splicing, is the key step affected by the presence of pol II.

This suggests that pol II, while it is not part of the splicing machinery



DOI: 10.1371/journal.pbio.0040183.g001

RNA synthesis by Pol II promotes the recruitment of the spliceosome to exon/intron junctions. The rapid association of RNA processing factors with the pre-mRNA prevents its premature degradation.

per se, somehow increased the ability of the splicing machinery to find and latch on to the pre-mRNA, thereby preventing RNA degradation and ultimately promoting splicing. They found further support for this conclusion by showing that functional splice sites on the pre-mRNA were needed for this effect to occur. Finally, they built a mathematical model of the entire range of RNA interactions, from synthesis to splicing or degradation, and showed that the key variable in determining the relative concentrations of the various RNA species was the affinity of the splice machinery for the pre-mRNA, highlighting the importance of this step in controlling the fate of the RNA.

This study did not address whether pol II and splicing are functionally linked—that is, whether some structural property or dynamic activity of pol II is coupled with the splicing machinery, or, instead, whether the mere proximity of the two ensures efficient transfer of RNA from one to the other. But other studies have highlighted the importance of pol II's C-terminal domain. Located at one end of this molecular behemoth, this region appears to promote other RNA processing events, including addition of the protective cap, and some splicing factors are known to associate with this domain. Further investigation of these interactions may reveal just how intimately linked transcription and splicing are.

Hicks MJ, Yang CR, Kotlajich MV, Hertel KJ (2006) Linking splicing to Pol II transcription stabilizes pre-mRNAs and influences splicing patterns. DOI: 10.1371/journal.pbio.0040147

Bacterial Symbionts May Prove a Double-Edged Sword for the Sharpshooter

Liza Gross | DOI: 10.1371/journal.pbio.0040218

Though scientists recently downsized estimates of insect species from over 30 million to around 4 million to 6 million, it's safe to say that insects are among Earth's most numerous and diverse group of organisms. At least some of this success derives from mutually beneficial interactions with bacteria, established as long as 270 million years ago, that allow insects to thrive in otherwise unsuitable niches. These symbiotic bacteria—called *endo*symbionts because they live inside cells—synthesize essential nutrients for their hosts, while the insects provide an ecological niche for the bacteria.

In a new study, Dongying Wu, Jonathan Eisen, Nancy Moran, and colleagues used comparative genome analysis to investigate the inner life of a widespread agricultural pest, the glassy-winged sharpshooter *(Homalodisca coagulata)*. As the vector of Pierce disease (caused by the *Xylella fastidiosa* bacterium), the sharpshooter poses a major threat to California crops, having already destroyed an estimated $14 million of Southern California grapevines. It lives on xylem sap—which distributes salts and water throughout the plant but provides very few organic nutrients to the insect—and houses two

DOI: 10.1371/journal.pbio.0040218.g001

The genomes of two bacterial species that supply essential nutrients for the glassy-winged sharpshooter provide potential targets for controlling infestations of this greatly feared agricultural pest

endosymbionts, *Baumannia cicadellinicola* and *Sulcia muelleri*. These two unrelated endosymbionts, they show, play distinct, complementary roles that supplement their host's diet.

Endosymbionts were first described through microscopy in the early 20th century, but their metabolic secrets have only recently come to light by making inferences about gene function based on genome analysis. After sequencing the *B. cicadellinicola* genome, Wu et al. built a genome-based evolutionary tree (called a phylogenetic tree) for *B. cicadellinicola* and related endosymbiont species. *B. cicadellinicola* was grouped together with endosymbionts of aphids *(Buchnera)*, tsetse flies *(Wigglesworthia)*, and ants *(Blochmania)*, in keeping with other genomic studies, but was "the deepest branching symbiont," suggesting it had separated from the group earlier.

The endosymbiont genomes shared a number of features, including reduced genome size, fewer guanine-cytosine (G-C) than adenine-thymine (A-T) base pairs, and rapidly evolving proteins. These trends can be found in endosymbionts arising across all the major bacterial groups, challenging biologists to figure out the mechanisms driving them. One explanation centers on changes that arise by chance in small populations (called drift); the other centers on a higher rate of mutations stemming from the loss of DNA repair systems. Interestingly, though some researchers favor one hypothesis over the other, analysis of the *B. cicadellinicola* genome suggests both are important, but act at different levels. The differences between endosymbionts and free-living species appear to be due to genetic drift. Differences among symbionts, however, appear to be due to differential loss of DNA repair genes.

The researchers argue that the nature of some of *B. cicadellinicola*'s other genome features (such as G-C content) and position on the evolutionary tree can help fill in missing gaps between free-living and intracellular species. And because its proteins are evolving more slowly than those of other endosymbionts—making it more likely that similar sequences really do reflect evolutionary relatedness rather than artifacts that occur when only fast-evolving sequences are used—inferences about the evolutionary events promoting intracelluarity can be made with more confidence.

As for *B. cicadellinicola*'s metabolic capabilities, the researchers found a "relatively limited repertoire." It lacks the necessary genes to support sugar metabolism, and probably does not use sugar as energy source since

almost none is present in the xylem sap diet. Energy likely comes from using the more abundant amino acids, one of the main types of organic compounds present in xylem. In return, it provides the insect with a range of vitamins and cofactors (required for enzyme activity). But surprisingly, *B. cicadellinicola* lacks key enzymes needed to synthesize essential amino acids. Obviously, the insect must get the missing essential nutrients from somewhere, and the most likely candidate, they reasoned, was the other bacterium known to reside in its cells, *S. muelleri*.

They went back to the sequences that didn't assemble with *B. cicadellinicola*, realizing they might contain part of the *S. muelleri* genome, and found genes required for the synthesis of several essential amino acids. Though some of the sequences belonged to other bacterial species, the vast majority required for amino acid synthesis belonged to *S. muelleri*. Since the sample of this bacterium's genome contained very few genes related to vitamin or cofactor synthesis, Wu et al. concluded that the two endosymbionts perform nonredundant, complementary services for their host. *B. cicadellinicola* synthesizes most of the sharpshooter's vitamins and cofactors, while *S. muelleri* appears to provide essential amino acids.

How three unrelated organisms manage to integrate such complex operations as metabolism and gene expression is a question for future study. But targeting any number of these essential bacterial pathways could prove a promising strategy for containing the spread of a potentially devastating pest—a risk perceived as so great that the sharpshooter is the only insect listed as a potential bioterrorism agent. And the researchers make a strong case that endosymbiont systems can help shed light on the molecular and evolutionary changes that allowed free-living organisms to take up residence in the cells of others—a path echoing the evolution of eukaryotes, whose cells contain mitochondria and chloroplasts, the descendants of ancient free-living bacteria.

Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL, et al. (2006) Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. DOI: 10.1371/journal.pbio.0040188

A Tiny Protein Plays a Big Role in DNA Repair

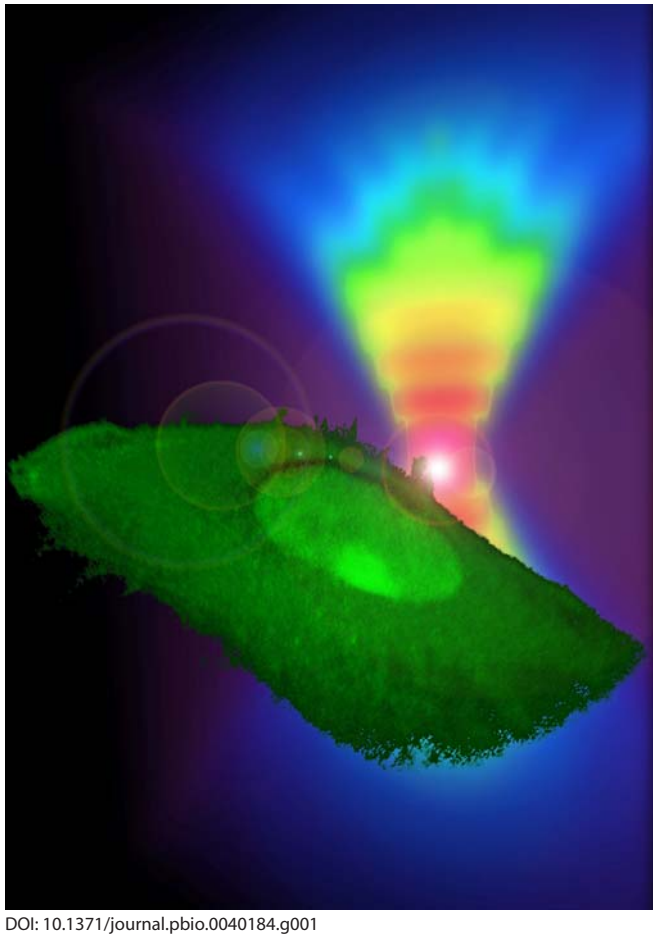
Liza Gross | DOI: 10.1371/journal.pbio.0040184

The human genome lies deep within the cell interior, sequestered behind the double-walled membrane of the nucleus, yet still remains vulnerable to a wide variety of hazardous materials. Two of DNA's worst enemies, ultraviolet light and chemical carcinogens, can wreak havoc on the molecule by mutating individual nucleotides or changing its physical structure. In most cases, genomic integrity is restored by specialized suites of proteins dedicated to repairing specific types of injuries.

One mending mechanism, called nucleotide excision repair (NER), recruits and coordinates the services of roughly 25 proteins to recognize and remove structure-impairing lesions, including those induced by ultraviolet light. At the center of this effort is the ten-subunit transcription/repair factor IIH (TFIIH) complex. As its name suggests, in addition to its role in DNA repair, it also regulates transcription; how TFIIH coordinates these very different activities is still under investigation. Regardless, three TFIIH genes—*XPB, XPD*, and *TTDA*—have been implicated in the photosensitive form of a rare inherited premature aging syndrome called trichothiodystrophy (TTD), which is characterized by brittle hair and nails, scaly skin, and neurological degeneration.

In a new study, Giuseppina Giglia-Mari, Jan Hoeijmakers, Catherine Miquel, Wim Vermeulen, and colleagues created a fluorescently tagged version of trichothiodystrophy group A (TTDA) to investigate its role in repair and transcription. By experimentally modifying the transcription function and by triggering DNA repair in human cell lines expressing the fluorescent TTDA protein, the researchers show that TTDA first of all dynamically associates with TFIIH, and that TTDAs become stably incorporated only while the complex is engaged in NER.

NER steps in when damage interferes with the elongation of a newly transcribed gene, promoting cell survival. It also plays an anti-cancer role by surveying the genome for damage. In ultraviolet-induced NER, TFIIH unzips the



DOI: 10.1371/journal.pbio.0040184.g001

Fluorescence accumulation in the nucleus indicates TTDA retention during the repair reaction at a locally ultraviolet-irradiated area in a living human fibroblast in this three-dimensional reconstruction. (Image: Pierre Olivier Mari and Giuseppina Giglia-Mari)

double helix to access the injury, and then recruits four proteins to stabilize the open strand, evaluate the damage, and cleave the DNA on either side of the lesion. Other proteins produce new nucleotides to fill the gap. Cells collected from patients with TTD-A have *TTDA* mutations that produce either truncated, nonfunctional versions of the protein or no protein at all, resulting in reduced TFIIH levels. TTD-A cells also have NER defects and are hypersensitive to ultraviolet light.

To study the role of the TTDA subunit in the TFIIH complex's functions, the researchers tagged TTDA and the XPD subunit with green fluorescent protein (GFP) to monitor and compare their movement and behavior using high-resolution confocal microscopy. Both TTDA-GFP and XPD-GFP could stably incorporate into TFIIH and function in DNA repair, proving reliable tools for studying TFIIH's spatial and temporal distribution and activity during transcription and DNA repair.

Both TTDA-GFP and XPD-GFP were observed in the cytoplasm and nucleus, in contrast to the XPB subunit, which is known to localize only in the nucleus. To determine if TTDA and XPD assemble with TFIIH in the cytoplasm, the researchers used a customized version of a motility-monitoring technique called fluorescence recovery after photobleaching (FRAP). Applying a high-intensity laser to a specific region in a cell destroys fluorescence in that area; as unaffected GFP-tagged molecules from other regions replace the zapped molecules, fluorescence is recovered. Recovery is much faster for free-moving GFP-tagged proteins than for those caught up in interactions with other cellular proteins.

In the cytoplasm, TTDA-GFP moved much faster than XPD-GFP, but did not interact with each other. Both proteins, however, were less motile in the nucleus, where they interacted with TFIIH. These results indicated that TTDA exists in two pools in non-irradiated cells, one free and one bound to TFIIH. After irradiation, however, fluorescence was not completely recovered for either TTDA-GFP or XPD-GFP, suggesting a lack of motility one would expect by participating in DNA repair. When the researchers inhibited a protein essential for NER initiation in the irradiated cells, they observed a reduced fraction of immobilized TTDA-GFP, confirming that TFIIH must incorporate TTDA-GFP (thus, reducing its mobility) for NER. By interfering with the transcription machinery, they go on to show that TFIIH does not stably incorporate TTDA when TFIIH is simply bound to DNA; TFIIH only enhances association with TTDA during NER.

Altogether, these results show that NER-specific lesions induce TTDA to form a stable association with the TFIIH in living cells. Giglia-Mari et al. propose that once TTDA nestles into place after the core TFIIH complex attaches to a lesion, it triggers a conformational change that recruits the other subunits required for repair. TTDA may also help TFIIH fold properly, preventing it from being degraded and allowing it to accumulate to the levels necessary for NER function. TFIIH molecules spend far longer at NER sites (four to five minutes) than they do at transcription sites (two to ten seconds), suggesting why TFIIH concentrations are so important to NER—and why people that can't produce TTDA experience such debilitating symptoms.

Giglia-Mari G, Miquel C, Theil AF, Mari PO, Hoogstraten D, et al. (2006) Dynamic interaction of TTDA with TFIIH is stabilized by nucleotide excision repair in living cells. DOI: 10.1371/journal.pbio.0040156

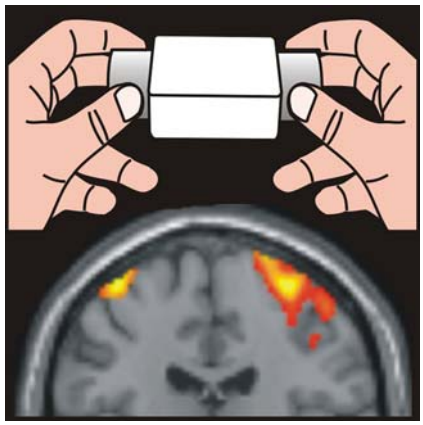
Making Hands Jive: How the Body Manages Hand Coordination

Mason Inman | DOI: 10.1371/journal.pbio.0040196

Unscrewing the lid of a honey jar stuck tight takes more than simply muscle power and a vice-like grip. It also takes coordination between your hands—one twisting the lid and the other holding the jar—to open the jar without it spinning out of your grasp.

But you might not suspect that in tasks like this, you unconsciously appoint one of your hands the leader and assign the other a supporting role—and the leader is not necessarily your writing hand. A new study reveals that people are surprisingly flexible in assigning these roles, and that they can quickly switch dominance between hands depending on the nature of the task. This dominance turns out to be thoroughgoing, from the brain's cortex down to specific muscles in each hand.

In the new experiments, Roland S. Johansson and colleagues had 37 people, all right-handers, play a video game similar to the arcade classic Whac-A-Mole: when a target appeared in a random spot on a video screen, the players moved a cursor to touch the target as quickly as possible. Key to the experiments was a custom-made controller, a rectangular box with knobs on two ends, that the players used. To move the cursor horizontally on the screen, the players pushed the controller's knobs inward or pulled them out; twisting the knobs, like screwing or unscrewing a jar's lid, sent the cursor up or down.



DOI: 10.1371/journal.pbio.0040196.g001

When the rules of a bimanual task changed, the center of activity switched between hemispheres in motor-control areas in the brain's cortex and cerebellum.

With this setup, the researchers could see whether a person's hands were acting perfectly in concert or whether one hand took a dominant role, acting a bit before the other or pushing or twisting harder than the other. The researchers found that one hand did consistently dominate, so the body of the controller would shift or twist slightly at first, before the supporting hand had a chance to compensate.

But which hand was dominant depended on the rules of the game, and the players were flexible, quickly switching dominance between their hands when the rules of the game

changed. For example, when the game was set up so squeezing the knobs inward moved the cursor to the left, then the players would make their right hand dominant. Conversely, when the researchers changed the rules so an inward squeeze sent the cursor right, then the left hand was dominant.

Johansson and colleagues found that the dominance of one hand manifests differently in each half of the body depending on the rules of the game. In the dominant hand, the muscles were stiffer and contracted at a higher rate, whereas the supporting hand was more relaxed and could compensate for the forces from the dominant hand. Also, functional magnetic resonance imaging (fMRI) showed that in motor-control areas of the brain's cortex and cerebellum, the center of activity switched between the brain's hemispheres when the rules of the game changed. Finally, the researchers used transcranial magnetic stimulation (TMS), bursts of magnetic fields that can boost activity in a specific brain region, to study how the brain's signals reached the hands. They found that the corticospinal pathways, which connect the brain's primary motor cortices with the spinal cord, were especially active only on the side controlling the dominant hand.

Together these findings suggest the body has a flexible system for assigning dominance to one hand while suppressing activity for the other hand so it can assume a supporting role, the authors argue. Dominance could come from the top down, from goals processed in the higher levels in the brain, or from the bottom up, perhaps from tactile sense of objects—or from a combination of the two. What's clear, however, is that the hands don't have to fight it out.

Johansson RS, Theorin A, Westling G, Andersson M, Ohki Y, et al. (2006) How a lateralized brain supports symmetrical bimanual tasks. DOI: 10.1371/journal.pbio.0040158