

Synopsis of Research Articles

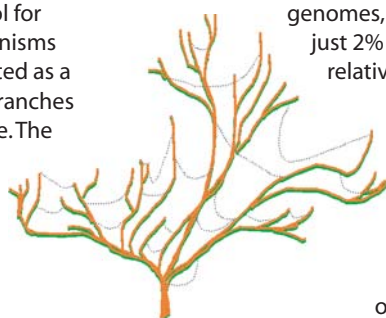
Comparing Gene Trees and Genome Trees: A Cobweb of Life?

DOI: 10.1371/journal.pbio.0030347

The tree of life has long served as a useful tool for describing the history and relationships of organisms over evolutionary time. One species is represented as a branching point, or node, on the tree, and the branches represent paths of descent from a parental node. The tree diagram carries an implicit assumption that genes are transferred vertically, from parent to child, and that all the genes in a new species come from the ancestral species. In theory, one should be able to trace the origin of each gene in a species back to its ancestor. In practice, however, the ancestral gene is rarely available, so researchers look for the gene in a closely related species. (These similar genes, which diverge slightly after a speciation event, are called orthologs.)

But as the tools of genome analysis became more refined, searches for similar genes sometimes turned up sequences that belonged to a species on a different branch of the evolutionary tree. Clearly, vertical gene transfer was not the only mechanism of genetic transmission. Organisms, it turns out, can acquire genes from non-ancestral species through a mechanism called horizontal gene transfer (HGT)—think of it as acquiring genes from your neighbor instead of your parents. Such genetic exchanges, most common among bacteria and other microbes, are not represented in the tree of life—no single branch connects the two unrelated species. Initial studies suggested that HGT events were extremely common, prompting some to say it was time to replace the tree with a netlike diagram. Other studies have since suggested that methods used to calculate HGT overestimated its frequency: researchers detect HGT events by finding inconsistencies between gene trees and organism, or whole-genome, trees, but statistical errors can artificially increase the number of HGT events.

In a new study, Fan Ge, Li-San Wang, and Junhyong Kim estimate the frequency of HGT events by using a novel statistical method to compare the gene trees and whole-genome trees of microbes. Their method solves the statistical problem by directly testing for discrepancies between trees that arise from statistical error versus true HGT events. Analyzing over 40 microbial



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Horizontal gene transfers—gene exchange between non-related organisms—appear commonplace among bacteria, but contribute just small bits of genetic information, leaving the traditional tree of life intact.

genomes, Kim and colleagues estimate that HGT infiltrates just 2% of the average microbial genome. Even when relatively common, the authors conclude, HGT events do not disrupt the integrity of the tree of life, contributing just small bits of genetic material, “much like cobwebs on tree branches.”

To construct both gene trees and a whole-genome tree for the microbes, the authors selected core sets of orthologous gene groups from the NIH database of clusters of orthologous genes. (Clusters are derived by comparing protein sequences encoded in complete genomes, which represent major lineages on the evolutionary tree. Each cluster corresponds to an ancient, conserved protein domain.) Kim and colleagues created gene trees for each cluster of orthologous genes they selected, then created whole-genome trees from the gene trees and compared each gene tree to the whole-genome tree, using their new

method. HGT events were detected when two species appeared close together on a gene tree but far apart on the whole-genome tree. Overall, just over 11% of the orthologous gene clusters showed statistically significant HGT events, with HGTs accounting for about 2% on average of each of the 40 microbial genomes.

Altogether, these results suggest that HGT is not as common as once thought. And even when large-scale HGT events do occur—which Kim simulated in a previous study—they do not obscure the evolutionary path of most genes and lineages. If you imagine a tree with 10,000 taxa, the authors explain, and 1,000 HGTs per genome across all the taxa, the HGTs would form “extremely thin connections like cobwebs,” leaving the backbone of the tree intact. Infrequent though it may be, HGT likely has some impact on the evolutionary history of life—impacts that advances in genome analysis technology may help uncover.

—Liza Gross

Ge F, Wang LS, Kim J (2005) The cobweb of life revealed by genome-scale estimates of horizontal gene transfer. DOI: 10.1371/journal.pbio.0030316

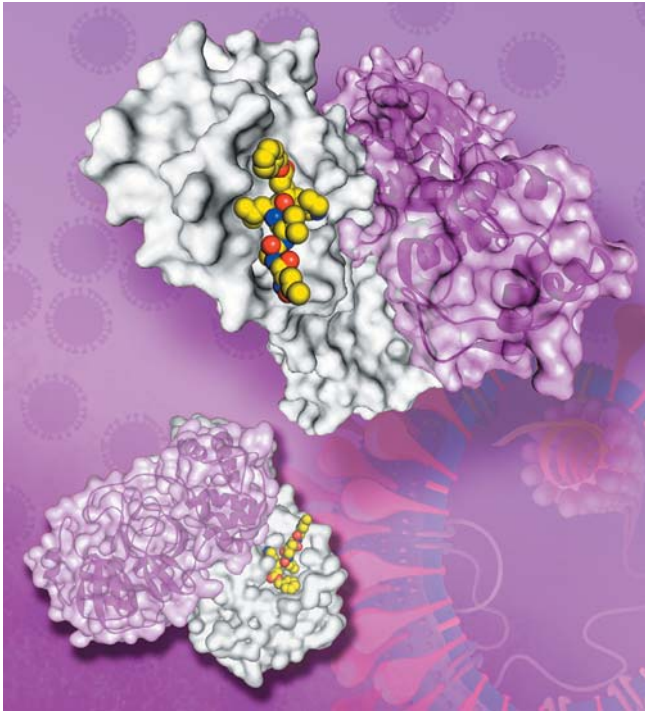
Casting a Wide Net to Fight Coronaviruses

DOI: 10.1371/journal.pbio.0030353

Viewed under a microscope, the coronavirus appears almost beautiful, thanks to the halo-like crown formed by its surface proteins. (“Corona” means “crown” in Latin.) Aesthetics aside, this genus of viruses is responsible for a wide range of animal and human diseases, from the common cold to the deadly severe acute respiratory syndrome, familiarly known as SARS. Research efforts to design antiviral agents to combat coronaviruses intensified after SARS killed at least 800 people in 2003 and have focused mostly on just this virus. But Haitao Yang, Dawei Ma, Zihao Rao, and colleagues reasoned that it might prove more efficient to develop wide-spectrum drugs and vaccines that could work against all coronaviruses—

significantly reducing the health and economic burden associated with the 25 species of coronavirus.

Scientists fear that vaccines may prove ineffectual against coronaviruses because the viruses, like HIV, change their protein sequences and structures so often that a vaccine targeting one strain would likely be ineffective against another. The success of such a vaccine strategy depends on finding a protein target that is present, or well conserved, among all the different coronaviruses. By combining structural and biochemical analyses, Yang et al. not only identified such a target in a conserved region of a viral enzyme but also designed compounds with antiviral activity against multiple coronaviruses.



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A broad-spectrum inhibitor that can recognize the active site of a coronavirus enzyme called the main protease could lead to the discovery of a single agent against coronaviruses. The protease structure is shown here in ribbon-and-surface representation; inhibitor molecules are the yellow, blue, and red balls.

Because coronavirus species show great diversity among their structural proteins—which include the glycoproteins that form the halo—the authors turned to three enzymes as potential targets. But since structural data were available for only one of the enzymes, called the main protease (M^{Pro}), the authors focused on M^{Pro} . Having structural data in hand greatly accelerates drug development, and since humans and other animals have no proteins similar to M^{Pro} , the likelihood of deleterious side effects is low.

Initial computer analysis showed that the M^{Pro} primary protein sequences (the linear amino acid sequences) have only 38% sequence identity between coronavirus species in some cases. But because three-dimensional structures tend to be more conserved than amino-acid sequences, the authors chose representative viruses from each group of coronaviruses to study and compare the structure of their M^{Pro} . This protease normally binds to its target protein (called the substrate) via a specific region, called the substrate-binding site. Structural analysis determined that this site is well conserved among coronaviruses, and biochemical tests confirmed that it would make a promising target for antiviral agents.

To test this hypothesis, the researchers created a synthetic version of the substrate that normally binds to the protease's substrate-binding site—reasoning that if they could inhibit the substrate's access to the binding site by the mimic (known as suicide inhibitors), they should be able to block the protease's activity and maybe halt viral replication. By studying the structure of the protease–substrate/inhibitor complex, Yang et al. continually improved their synthetic inhibitor until it bound strongly to the protease. Using this initial inhibitor as a base, the authors designed a panel of inhibitors and identified compounds that rapidly blocked proteases from multiple coronaviruses and kept the coronaviruses from reproducing. The compounds caused no obvious damage in human cells in the experiments.

The substrate-binding site identified by the researchers is an especially attractive target for drug development because evolutionarily conserved regions do not undergo high mutation rates like the rest of the viral genome, allowing antiviral drugs to maintain their effectiveness. Support for this hypothesis comes from the finding that a compound developed in this study also inhibits M^{Pro} from new coronavirus strains that cause conjunctivitis, bronchiolitis, and pneumonia. By identifying promising candidates for drugs capable of targeting the entire *Coronavirus* genus, Yang et al. have laid the foundation for containing everything from the common cold to the deadly SARS virus. Preclinical and clinical trials will show whether these compounds live up to their promise. —*Supriya Kumar*

Yang H, Xie W, Xue X, Yang K, Ma J, et al. (2005) Design of wide-spectrum inhibitors targeting coronavirus main proteases. DOI: 10.1371/journal.pbio.0030324

The Molting Worm Sheds Its Genetic Secrets

DOI: 10.1371/journal.pbio.0030345

Anyone who winces remembering the challenges of childhood might consider the fate of the developing worm. Four times in its life, a growing nematode worm flips on its side and writhes around to shed its exoskeleton, or cuticle. During each molt, a worm casts aside its cuticle and synthesizes a new protective shell, its primary defense against a harsh environment. Though it's clear that a complex array of signaling proteins and enzymes are required to engineer these rites of passage, only a few genes have been implicated in the process.

Scientists have found critical molting genes in the fruitfly, but most of these

are not present in the worm. It may be that the worm has unique molting genes, because its cuticle is more elastic than the hardened casing of an insect. If scientists find molting genes that exist only in the worm, they can begin to unravel the mechanisms that govern these critical phases of a worm's life. They can also develop treatments that target the worm's parasitic cousins—which wreak havoc on humans, livestock, and plants—without producing harmful side effects. In a new study, Alison Frand, Sascha Russel, and Gary Ruvkun searched the entire genome of the worm *Caenorhabditis elegans* for molting genes and identified 159

candidates, using a technique called RNA interference.

In RNA interference, researchers use double-stranded RNA (dsRNA) to block the expression of, or silence, a specific gene by destroying the gene's messenger RNA transcript before it can be translated into protein. These dsRNAs can be expressed in bacteria—a staple food for lab worms—which then multiply into large colonies expressing the same dsRNA. Using a pre-existing “library” of bacterial clones that each express a particular dsRNA, the authors fed groups of larvae one bacterial clone at a time—until thousands of larvae had eaten bacteria with dsRNA designed to

silence nearly every one of the worm's 19,427 genes.

After the larvae ate the bacterial clones, the authors screened them for molting defects—which is how they identified the 159 genes. Molting defects mostly left larvae trapped in their old cuticle; those that managed to escape often failed again during the next molt. The majority of candidate molting genes appear to play a role in all four molts, the authors argue, since their inactivation foils molting at several stages. And, significantly, the majority of genes—many of which are found only in worms—exist in worm parasites that infect humans, animals, and plants.

Among the genes identified, the authors found several transcription factors (proteins that activate genes), indicating that molting requires “extensive changes in gene expression.” Other genes are associated with signaling proteins that likely coordinate the activity of different cell types during molting, and many genes code for proteins that are required for protein synthesis—likely to build the



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As this *Caenorhabditis elegans* larva molts between developmental stages, green fluorescent protein allows researchers to trace the expression of one of its molting genes (*mlt-11*). Defects in this gene trap larvae in their cuticle.

new cuticle. Still other genes may help remodel the cuticle.

To monitor the expression of some of these genes and infer their function, the authors tagged a subset of genes—representing many of the functional categories found in the screen—with

green fluorescent protein. Since green fluorescent protein glows when a gene is activated, the researchers can see where and when genes are expressed. Fluorescence levels were high just before each molt and dropped off soon after. All of these genes were expressed in the epithelial cells that secrete new cuticle. These results provide strong evidence for the genes' role in molting, since they were expressed both at the right time and the right place. These experiments also allowed Frand et al. to propose a model describing the timing and order of gene expression during molting.

With all the genes identified in this screen, researchers can now start to piece together the overlapping pathways that guide the worm through its formative years. And with the discovery of worm-specific genes, it's likely that more effective treatments await patients with elephantiasis, African river blindness, and other diseases caused by pathogenic nematodes. —Liza Gross

Frand AR, Russel S, Ruvkun G (2005) Functional genomic analysis of *C. elegans* molting. DOI: 10.1371/journal.pbio.0030312

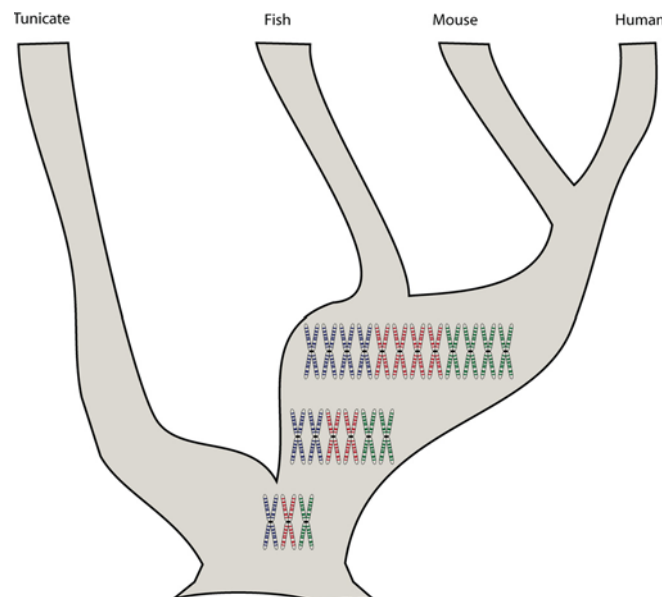
Clear Evidence for Two Rounds of Vertebrate Genome Duplication

DOI: 10.1371/journal.pbio.0030344

As one of the most important sources of novel gene functions, gene duplications play a major role in evolutionary change. Though a gene copy will generally become inactive after duplication, it can be saved—either by acquiring a new function or dividing aspects of the original gene's function—on its way to becoming ubiquitous, or “fixed,” within the population.

The notion of “evolution by gene duplication” was proposed in 1970 by Susumu Ohno, who argued that gene and whole genome duplication provided the raw material for evolutionary innovations such as subcellular compartments, fins, and jaws. Having “extra” copies of genes provides the opportunity for duplicate genes to escape the constraints of purifying selection, and allows the genes to diverge and acquire novel functions. Ohno also proposed that two rounds of whole genome duplication occurred at some point in early vertebrate evolution—a possibility that could explain the relatively large size and complexity of the vertebrate genome.

Investigators equipped with far more powerful genome-mining tools than were available to Ohno have long sought evidence of this hypothesis (known as the 2R hypothesis, for “two rounds” of whole genome duplication), but with conflicting results. The observation that some gene families have four members in vertebrates but just one in invertebrates (the 4:1 rule) appeared to support the 2R hypothesis, until it was discovered that less than 5% of homologous gene families (similar genes with shared ancestry) followed the rule. But even when gene families do follow the rule, their configuration could



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The evolutionary patterns of gene duplications were reconstructed by comparing the complete gene sets of a tunicate (sea squirt), fish, mouse, and human. The 4-fold pattern in their global physical organization provides unmistakable evidence of two distinct, ancient whole genome duplications.

just as likely arise from two rounds of single gene duplication as from whole genome duplication. And because duplicate genes are far more likely to degrade than to assume new or shared functions, the signal of whole genome duplication disappears.

Recent studies have shown that the global pattern of the physical location of homologous genes provides evidence of ancient whole genome duplications in yeast and plants, even when most of the duplicates have degraded. Now Paramvir Dehal and Jeffrey Boore have taken this approach to test the 2R hypothesis, by comparing the recently completed genome sequence of the invertebrate sea squirt with the genomes of three vertebrates—pufferfish, mouse, and human. (Because the sea squirt is a close relative of vertebrates, its genome can help reconstruct a more accurate tree of the organisms' evolutionary relationships than a more distant relative like the fruitfly could.)

After generating gene clusters that each contained "all, and only, those genes that descended from a single gene in their common ancestor," the authors used a method to infer the evolutionary relationships of the genes in each cluster. They could then compare these gene trees to the known evolutionary relationships of the organisms to determine when each gene duplicated in relation to when the lineages diverged. From this analysis, Dehal and Boore identified over 3,500 gene duplications present in multiple vertebrates, indicating they had occurred at the base of the vertebrate tree, dating back some 450 million years. But did these early duplication events arise from some large-scale duplication event, or were they simply the result of a great number of smaller scale duplications?

To explore this question, the authors analyzed the relative positions of the resulting paralogs in the vertebrate genome with the highest-quality data—the human genome. When considering only this subset of 3,500-plus early vertebrate duplications, they found a global pattern of human genome segments with similar arrangements of paralogous genes and multiple chromosomes with long linear stretches of interdigitated sets of paralogous genes—evidence that the duplications occurred in large segments. Even stronger support for the 2R hypothesis comes from the observation that the colinear arrangement of these genes is predominantly in a 4-fold pattern; this repetitive pattern is seen across almost all the human chromosomes. It's unlikely, the authors argue, that any combination of smaller, independent duplication events could have generated the same pattern.

Now that strong evidence for Ohno's hypothesis exists, researchers can investigate both the mechanism of genome duplication events and their possible effects on vertebrate evolution. It seems likely that a whole genome duplication would provide combinatorial possibilities that could permit a greater leap in evolution than could single gene duplications, even if the single gene duplications affected the complete set of genes. Studies that examine the function of these paralogous genes can explore whether these large-scale genomic events helped drive organismal complexity and diversification within the vertebrate lineage. —*Liza Gross*

Dehal P, Boore JL (2005) Two rounds of whole genome duplication in the ancestral vertebrate. DOI: 10.1371/journal.pbio.0030314

Clues to the Evolution of the Malarial Chromosome

DOI: 10.1371/journal.pbio.0030361

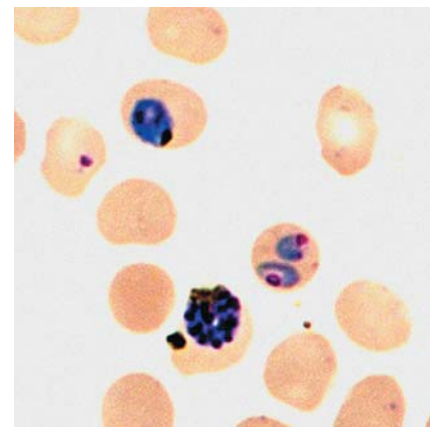
Understanding the recombination patterns across a chromosome—determining the positions and frequency of genetic exchanges between homologous chromosomes—is crucial for understanding and tracking inheritance of traits. Mapping genes that affect parasites' traits, such as responses to various antimalarial agents, is possible because, during meiosis, homologous chromosomes line up and may exchange segments. Genes—or any polymorphic bits of DNA—that are close together tend to remain linked during this process, while those far apart tend to become separated. Identifying and following polymorphic markers through multiple generations is a key technique for genetic mapping.

For *Plasmodium falciparum*, the microbe that causes malaria, chromosomal mapping is necessary for understanding the evolution of the parasite and development of drug resistance, but multiple factors make this a complex task. In this issue, Jianbing Mu and colleagues use single nucleotide polymorphisms (SNPs) to evaluate some of these factors, and set the stage

for further mapping of this important parasite's genome.

The authors began by locating 183 SNPs spaced across Chromosome 3 in 99 *P. falciparum* populations from throughout the world. Not all SNPs were found in all populations, indicating a more recent evolutionary origin for some SNPs; these differences were then used to track evolution and migration in parasites. Statistical analysis of the SNPs allowed the populations to be parsed into five groups, largely corresponding to continents. More refined analysis of the SNPs revealed possible migratory history, including a recent migration of an African variety to coastal South America.

Mu and colleagues also showed for the first time that the historical rate of recombination varies widely—over 20-fold—among different populations. A large part of the variation is due to a combination of the frequency of infections with multiple parasite strains (because sexual recombination occurs only within an infected mosquito) and the degree of inbreeding within a parasite population. Inbreeding tends



DOI: 10.1371/journal.pbio.0030361.g001

***Plasmodium falciparum*, the microbe that causes malaria, infects red blood cells. By analyzing different populations of the pathogen from around the world, researchers found clues to its genome structure that will be important for identifying genes that contribute to drug resistance and virulence.**

to lower the extent of detectable recombination events, while multiple infections by different strains increase it.

Despite the wide differences in recombination rates, all populations had a similar clustering of recombination “hot spots” at the middle and ends of the chromosome. Recombination is most likely to occur at these spots, and the similar localization reflects either the common evolutionary history of all the populations or localization of crossover events to particular genomic regions.

The authors compared their results from population structure analysis with those

using SNPs from genes that might be influenced by drug pressure. Their results showed that misleading inferences about the parasite population structures could be derived using information from genes that are potentially under drug selection.

These results are important because they provide information on the multiple complex factors that must be considered in understanding the genomic structure of *P. falciparum*, which is critical for identifying genes that contribute to

phenotypes such as drug resistance and virulence. Researchers conducting future mapping studies will be able to draw on the important findings and caveats revealed by this work to refine their own methods and interpret their results.
—Richard Robinson

Mu J, Awadalla P, Duan J, McGee KM, Joy DA, et al. (2005) Recombination hotspots and population structure in *Plasmodium falciparum*. DOI: 10.1371/journal.pbio.0030335

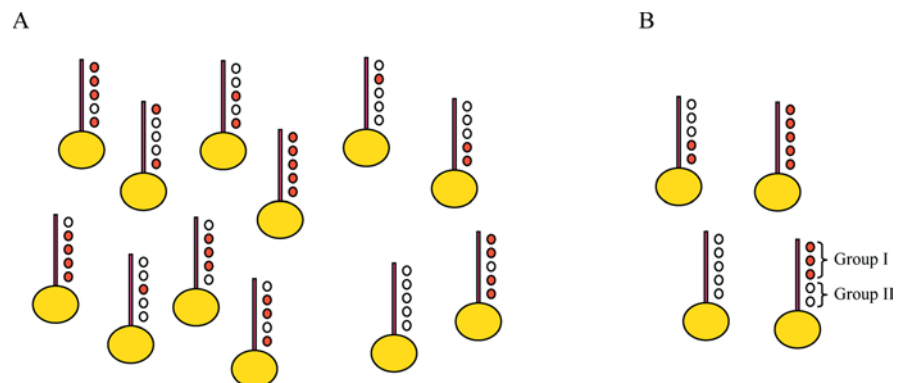
A Global View of DNA-Packing Proteins Cracks the Histone Code

DOI: 10.1371/journal.pbio.0030346

In one of biology’s most impressive engineering feats, specialized proteins package some six-and-a-half feet of human DNA into a nucleus that averages just 5 microns (0.0001969 inches) in diameter. In the first of a series of supercondensing steps, DNA winds around proteins called histones, which together form a complex called the nucleosome. Histones package DNA into repetitive coils, which not only provide genomic structure but also help regulate gene expression. These tasks are mediated in part by chemical modifications to histone proteins—most commonly to histone “tails,” long, unstructured chains of amino acids that protrude from nucleosomes. Different chemical modifications are associated with different functional effects. Acetylation, which adds an acetyl group to an amino acid on the histone tail, has been linked to both gene activation and silencing, depending on which amino acid is modified. Methylation (addition of a methyl group to the histone tail) has also been linked to gene activation and repression, although the chemical effects of methylation differ dramatically from those of acetylation.

Even in yeast, amino acid modifications in the histone tails can number in the tens and twenties. Given the number of possible permutations of modification types and amino acids, the question arose, might different combinations of histone modifications produce discrete outcomes? The notion that a sequence or combination of specific modifications on histone tails acts as a signal to other proteins and produces distinct biological effects was advanced as the “histone code” hypothesis in 2000.

Progress in deciphering the vocabulary, mechanics, and function of the histone code has been hindered by



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Histones can undergo many potential modifications, and it has been hypothesized that these can occur in many different combinatorial histone modification patterns (A). In this study, researchers found that only a few modification patterns occur in yeast, with many of the modifications co-occurring in groups (B).

the coarse resolution of available tools. Nucleosomes typically cover about 146 base pairs, but existing technology could only average over 500 to 1,000 base pairs at a time—confounding the effects of single nucleosomes. In a new study, Oliver Rando and colleagues take advantage of the high resolution afforded by their custom-made microarray, which has a resolution of 20 base pairs. Working with the budding yeast *Saccharomyces cerevisiae*, the scientists examined 12 different histone modifications in individual nucleosomes and found only a small number of distinct combinations with “few discrete histone modification patterns.” The concurrent modifications fall into two categories: one set targets a transcriptional start site but is the same no matter what the level of transcription, while the other occurs throughout gene coding regions and is linked to transcription. Importantly, the only modifications that appear to correlate with transcription occur over

transcribed regions, as though they were the consequence, rather than the cause, of transcription.

Why might histone tails exhibit so many modifications if they form only two independent categories? It’s possible that histone-modifying enzymes may work best in groups and so the marks that recruit them—acetyl and methyl groups—also come in groups. Another possible explanation relates to how histone modifications signal transcription enzymes that a particular gene requires more or less transcription. When the positively charged amino acid lysine acquires an acetyl group, it loses its charge, and charge–charge interactions play a major role in many interactions between proteins and other molecules. Multiple lysine acetylations on the histone tail may thereby aid certain chemical reactions necessary for transcription in a continuous way; having multiple levels of acetylation, for example, may allow the cell to “tune”

protein–protein interactions, and thus gene expression, up and down, rather than simply turn it on or off.

Rando and colleagues propose that the histone modifications associated with transcription may facilitate rather than trigger gene expression, perhaps by clearing a path for the transcription machinery or attracting proteins needed for the job. The authors are careful to point out, however, that histone modifications may also play some role in initiating gene expression, but that any transcription pattern would likely

be obscured, or “erased,” as transcription occurs. While future studies will help determine which role proves more common, these results suggest that histone modifications are facilitators rather than activators and that the histone code is more a transcription footprint than a starting signal. —*Liza Gross*

Liu CL, Kaplan T, Kim M, Buratowski S, Schreiber SL, et al. (2005) Single-nucleosome mapping of histone modifications in *S. cerevisiae*. DOI: 10.1371/journal.pbio.0030328

Complex Cells in the Brain’s Vision Center Tune in to Natural Scenes

DOI: 10.1371/journal.pbio.0030360

An Amur tiger roaming the snow-covered forests of the Russian Far East sees life differently than an arboreal primate raised in the thick canopy of a tropical jungle. Adaptations in the structure of the eye and the visual centers of the brain facilitate these different worldviews, fine-tuning each animal’s vision to the light levels and visual properties of its environment.

The notion that brain circuits are adapted to represent natural stimuli is known as the efficient coding hypothesis. In this framework, the visual system responds most effectively to the features found in the natural environment, like the specific arrangement of trees, another animal’s face, or the contrast of land abutting a river. Natural images possess different statistical features than random noise (such as static on a TV monitor), so responses to these two classes of stimuli could be distinct. According to the prevailing hypothesis, the retina and the lateral geniculate nucleus, the brain area that relays neural signals to the cortex, are tuned to the power spectrum of light signals found in natural scenes. In other words, these neurons are sensitive to the dominant energy at particular spatial or temporal frequencies in natural scenes and less sensitive to random noise, which has an overall flat power spectrum.

In a new study, Gidon Felsen, Yang Dan, and colleagues investigate how neurons in the cat primary visual cortex (V1) respond to images with natural statistics, and discover something truly novel about a V1 neuron’s sensitivity to features in natural scenes: a specific class of V1 neurons, called complex cells, are preferentially tuned to the phase regularities of light signals in natural scenes, not to the power spectrum.



DOI: 10.1371/journal.pbio.0030360.g001

By recording cortical responses to several classes of natural and synthetic images, researchers showed that complex cells are tuned to the phase structure of natural images to represent image features, such as the edges highlighted on the clock tower, efficiently.

(Phase relates to how the signals in different spatial frequencies align, which gives rise to edges in the image.)

The V1 contains two types of neurons, known as simple and complex cells, that were originally distinguished based on how they respond to light, determined by shining a flashlight on a wall and mapping the space that triggered neuronal activation. This activation space defines the cell’s receptive field. The receptive field properties of simple and complex cells vary significantly: simple cells behave linearly (two spots of light in the receptive field doubles the response), and complex cells behave nonlinearly.

To characterize the feature sensitivity of these neurons, the authors measured their response to different classes of

visual stimuli, including natural, random, and synthesized images; the synthesized images helped them distinguish between the power spectrum and phase effects. Feature sensitivity depends on a neuron’s preferred features, which the authors estimated from a neuron’s response to a set of natural images, including a man’s face, a building, a lion, and a hand. The authors created a set of random images with global and feature contrasts that matched the natural images, based on the preferred features, and recorded neuronal response to both the natural and random image sets. Overall, the majority of complex cells showed higher feature sensitivity for the natural stimuli, indicated by the contrast-response function, which plots neuronal response against the contrast of the feature. (A steep contrast-response function shows high neuronal sensitivity to a preferred feature, while a flat function indicates insensitivity.) Simple cells showed no difference in their response to natural and random stimuli.

Since the contrasts of natural and random image sets were matched, the complex cells’ sensitivity to natural images could not be explained by differences in overall contrast. More likely, the authors reasoned, the cells were responding to the power or phase spectra of the light. Felsen et al. manipulated each property in synthesized image sets to distinguish their effects on neuronal response. In one image set, each image had a natural power and random phase spectrum, while a second image set had the reverse. Comparing the feature sensitivity of complex cells to each of these image sets with a random image set, the contrast-response function, and thus feature sensitivity, was highest for the synthesized natural phase image set.

By experimentally linking visual statistics with neuronal responses, this study not only reveals a novel coding response property of complex cells but also provides evidence for the theory of efficient coding. The finding that complex cells selectively respond to properties of natural stimuli that simple cells don’t shows how brain circuits divide tasks to make the most of available resources. Researchers can now investigate how the structure of complex cells engenders their heightened sensitivity. —*Liza Gross*

Felsen G, Touryan J, Han F, Dan Y (2005) Cortical sensitivity to visual features in natural scenes. DOI: 10.1371/journal.pbio.0030342

A Signaling Pathway at the Heart of Muscle Development

DOI: 10.1371/journal.pbio.0030358

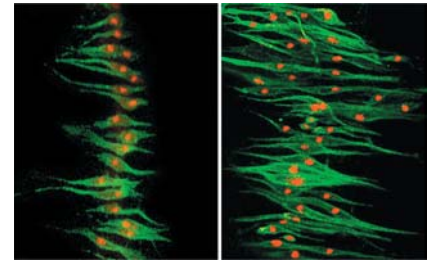
Unlike virtually every other type of cell, muscle cells contain dozens or even hundreds of nuclei. These multinucleate cells, called myofibers, form by fusion of precursor cells, called myoblasts, with “founder cells.” In the fruit fly, embryonic founder cells are formed by a well-known signaling pathway, but the same mechanism is not used to form adult founder cells. In this issue, K. VijayRaghavan and colleagues identify several key molecules involved in adult founder cell formation, and show that the process occurs through a novel mechanism.

In the fly embryo, founder cells differentiate from myoblasts through the actions of a membrane-bound receptor called Notch, an important player in several “signaling cascades” that use environmental signals to trigger changes in gene expression. However, in previous work, the authors have shown that Notch does not play a role in establishing adult founder cells. Instead, several clues pointed to the Fibroblast growth factor (FGF) family of receptors, one of which, in the fly, is called Heartless. Among other locations, Heartless is found on the surface of adult myoblasts in the abdomen. Reducing its expression in these cells, the authors showed, reduced the number of founder cells, while elevating it increased them.

But since Heartless is found in all adult myoblasts, it could not be responsible by itself for converting a

myoblast into a founder cell. Another protein, called Heartbroken, seemed like a good candidate, since it functions exclusively within the FGF pathway. The authors showed that while the gene for Heartbroken is initially expressed in all myoblasts, over time its expression becomes restricted to those cells that develop into founder cells. Furthermore, by artificially maintaining Heartbroken expression, the authors dramatically elevated the number of founders in developing muscle, strengthening the case that Heartbroken is a key promoter of founder cell development.

But since both Heartless and Heartbroken are initially present in early myoblasts, what prevents wholesale Heartless signaling and premature, widespread founder cell formation? The authors show that a third factor, called Sprouty, declines in expression as founders are specified, and is not detected after founders are established. Sprouty is known to be a negative regulator of FGF signaling. VijayRaghavan and colleagues suggest that Sprouty interferes with Heartless signaling in early myoblasts, preventing founder cell formation even in the presence of Heartbroken. The gradual decline in the level of Sprouty may then “release the brakes” on Heartless signaling. Not every myoblast becomes a founder cell at that point, though, because the level of Heartbroken has also declined. Exactly which cells will maintain sufficient Heartbroken to



DOI: 10.1371/journal.pbio.0030358.g001

***Drosophila* adult muscle founder cells are selected by the Heartless signaling pathway. Overexpression of components of the Heartless pathway increases the number of founder cells (right) compared to normal expression (left).**

become founders, and how those cells are specified, remains to be worked out.

Still more remains to be discovered about the development of muscle in the adult fly. The significance of this work lies in identifying the FGF pathway as a critical component in muscle cell development, which provides leads that can be used to fill in the missing members of the pathway. While the details of vertebrate muscle development differ, the FGF pathway is known to be involved there too, and this work may shed light on aspects of that process as well. —Richard Robinson

Dutta D, Shaw S, Maqbool T, Pandya H, VijayRaghavan K (2005) *Drosophila* Heartless acts with Heartbroken/Dof in muscle founder differentiation. DOI: 10.1371/journal.pbio.0030337

Mapping Core Communication Networks in Bacteria

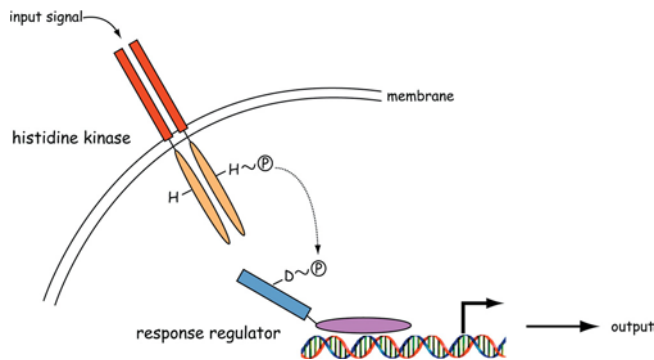
DOI: 10.1371/journal.pbio.0030359

Single-celled bacteria may appear simple by some standards, but these tiny cells employ sophisticated systems for processing stimuli. One especially important class of signaling molecules that help bacteria coordinate the activities of daily life is called the two-component signal transduction system. This system—comprised of enzymes called histidine kinases and their target molecules, the response regulators—allows bacteria to sense and respond to their surroundings by transforming various environmental cues, such as sugars, peptides, and antibiotics, into physiological responses. These environmental signals trigger a chemical reaction in histidine kinases called autophosphorylation, in which the kinase transfers a phosphoryl group from a molecule of ATP (which powers many cellular processes) to one of its own amino acids. The enzyme then transfers the phosphoryl group to its target response regulator, producing changes in gene expression, motility, protein breakdown, and various other cellular processes.

Though it's possible to identify histidine kinases and response regulators in bacteria by analyzing their genome sequences, it's far more difficult to determine how the two components interact: do they form monogamous pairs or behave more promiscuously? To characterize the range of possible interactions and the intracellular changes they bring about, Michael Laub and colleagues developed a novel method of mapping the connections between histidine kinases and response regulators in the freshwater bacterium *Caulobacter crescentus*. By combining genetic and biochemical analyses on a system-wide scale, the authors rapidly identified two-component signaling pathways in *C. crescentus*, including pathways required for core cell processes.

Laub and colleagues first identified 106 two-component signal transduction genes (62 histidine kinases and 44 response regulators) from the genome sequence. Then they created mutant strains of bacteria that each had one of the 106 genes





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In the two-component signaling pathway, a stimulus causes a histidine kinase to autophosphorylate on a conserved histidine (H) residue. The phosphoryl group (P) is then transferred to a conserved aspartate (D) residue of a cognate response regulator; when the response regular phosphorylates, it triggers a physiological response, such as gene transcription.

deleted (called deletion strains) to learn how the genes function. The phenotypes, or physical characteristics, of the mutant strains allowed the scientists to identify 39 genes required for cell cycle progression, growth, and morphogenesis, including nine genes essential to survival. To address the promiscuity question and figure out the likely phosphotransfer pairings among the components, the authors developed a biochemical method, called phosphotransfer profiling, that quickly identifies a histidine kinase target by tracking the transfer of radioactive (called radio-labeled) phosphates from the kinase to the target.

The authors validated their *in vitro* technique on two-component proteins from *E. coli* (a system in which many of the living bacteria's kinase-target pairings are known) by showing

that the kinases preferentially phosphorylated their known targets in the test tube as well, forming promiscuous unions only after prolonged periods. Confident that their method would also work for other bacteria, the researchers applied it to *C. crescentus* and determined the likely pairings for previously identified histidine kinases. Since the authors again observed a high preference for known targets, they were confident that these pairings were real, and the method could be used to identify targets of other, uncharacterized kinases. Based on the deletion analysis results, Laub and colleagues focused on a histidine kinase that appears essential for growth or survival and identified a single target response regulator. Eliminating the expression of each gene produced nearly identical phenotypes, revealing the pair's role in a signaling pathway that maintains the integrity of the bacteria's cellular envelope. Because two-component systems aren't found in humans, this cellular-envelope pathway may prove an effective antibiotic target against pathogenic bacteria, based on the effectiveness of other antibacterial therapies aimed at the cell membrane.

The library of deletion strains the authors developed will be a valuable resource, not only for identifying other two-component pathways in *C. crescentus* but also for studying aspects of the cell cycle and development, thanks to the bacterium's unusual life cycle—it divides asymmetrically, producing a motile daughter cell and a stationary one that can then differentiate into the motile version. And since the techniques presented here should work in any organism with two-component signaling (most bacteria, fungi, and many plants), researchers can apply them to the tall task of decoding the labyrinthine communication systems that sustain cellular life. —Liza Gross

Skerker JM, Prasol MS, Perchuk BS, Biondi EG, Laub MT (2005) Two-component signal transduction pathways regulating growth and cell cycle progression in a bacterium: A system-level analysis. DOI: 10.1371/journal.pbio.0030334

Field Tested: Grasslands Won't Help Buffer Climate Change as CO₂ Levels Rise

DOI: 10.1371/journal.pbio.0030329

Serious gardeners know a healthy harvest depends on the right mix of ingredients at the right time. Nitrogen, which spurs leafy growth at the expense of flowers, works best soon after plants emerge. Phosphate, which promotes blossoms, should come later. Indoor growers often gas their plants with CO₂ to boost growth, development, and budding. The *really* serious indoor gardener can pay \$599 for a microclimate controller that automatically regulates temperature, humidity, CO₂, lighting, and irrigation.

These components are just as important to natural ecosystems as they are to the well-tended garden. But their composition in the atmosphere and soil is changing at an unprecedented rate. Rising concentrations of CO₂ and other greenhouse gases—which have increased apace with global agricultural and industrial development—are linked to warmer temperatures and changes in precipitation patterns. The

by-products of industrial and agricultural operations have also increased nitrogen concentrations, saturating systems in highly polluted areas.

Plants use CO₂ (and water and light) during photosynthesis to make sugar and grow. A plant absorbs CO₂ through its leaves and takes up nitrogen (often in the form of nitrate) and other soil nutrients through its roots. Increasing CO₂ concentrations can facilitate plant growth by increasing the rate of photosynthesis, though this effect varies depending on the photosynthetic pathway the plant uses. Trees, for example, use a different pathway than most tropical grasses. Higher carbon inputs can also trigger more efficient nitrogen use. Because plants are primary producers, at the base of the food chain, major shifts in the global cycles that support them will have wide-ranging consequences.

To observe how a natural system might respond to these changes over

the long term, a team led by researchers from the Carnegie Institution of Washington and Stanford University created an experimental system in their Northern California backyard. Jeffrey Dukes, Christopher Field, and their colleagues treated grassland plots to every possible combination of current or increased levels of four environmental factors—CO₂, temperature, precipitation, and nitrogen influx—to simulate likely regional changes over the next 100 years. Previous studies have tested natural systems' responses to one or two of these changes, but none has tested the long-term, simultaneous impacts of each.

The strongest effects on grassland production came from elevated levels of nitrogen (which typically reaches a fertilization limit). Elevated temperature, precipitation, and, surprisingly, CO₂, had minimal impacts. These results suggest, the authors argue, that California grasslands, and ecosystems that respond



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Researchers work on some of the 36 plots that lie below four infrared heaters in the Jasper Ridge Global Change Experiment. Total plant growth in grassland plots like these rarely responded to changes in climate or atmospheric CO₂ concentration.

similarly, are not likely to help buffer the rate of climate change by acting as a carbon “sink”—slowing the rise of CO₂ levels by storing more carbon in new growth. It’s thought that ocean and terrestrial ecosystems have stored nearly half the carbon emissions produced by humans since the industrial revolution. If it turns out that other natural systems also fail to sequester as much carbon as scientists once thought, atmospheric CO₂ concentrations will rise even faster than expected—with serious implications for future climate change.

The experiments were part of the Jasper Ridge Global Change Experiment (JRGCE), which started on Stanford’s

1,200-acre biological preserve in 1997. Since 1998, this grassland ecosystem has been outfitted with an ecologist’s version of a microclimate controller (complete with CO₂ pumps, heaters, and irrigation tubing) and subjected to experimentally controlled atmospheric, climatic, and nutrient conditions. (This study examines the experiment’s first five years.) To quantify the grassland response to these treatments, the authors estimated net primary production, or NPP (the amount of carbon left over after cellular respiration) by measuring shoot and root growth in 36 circular plots scattered across roughly two acres. Four control plots

experienced the natural variations of California’s Mediterranean climate.

Overall, increased rainfall, warming, and elevated CO₂ had little effect on NPP. (More rain triggered shoot growth but stunted root growth, so NPP wasn’t affected.) In some experimental treatments and years, elevated CO₂ actually reduced grassland production. Increased nitrate, on the other hand, led to shoot and root growth imbalance, with shoots growing faster than roots. And this added nitrogen “strongly increased” NPP in every year but one. These results suggest that increasing concentrations of atmospheric CO₂ are not likely to increase growth of the roots and leaves of plants in this grassland. Why not?

One possibility involves phosphorus. High levels of CO₂ and nitrogen can reduce phosphorus concentrations or limit its uptake in these plants. Ongoing JRGCE experiments are exploring how this and other factors—such as grazing or shifts in seasonal events—might limit the growth effects of CO₂.

Because grasslands and forests operate in complex feedback loops with both the atmosphere and soil, understanding how ecosystems respond to global changes in climate and element cycling is critical to predicting the range of global environmental changes—and attendant ecosystem responses—likely to occur. Ecosystem responses may well vary according to their composition and location. By studying natural systems over multiple years on an ecosystem scale, experiments like JRGCE offer a valuable tool for simulating predicted global changes and assessing their likely impacts, region by region. —Liza Gross

Dukes JS, Chiariello NR, Cleland EE, Moore LA, Shaw MR, et al. (2005) Responses of grassland production to single and multiple global environmental changes. DOI: 10.1371/journal.pbio.0030319

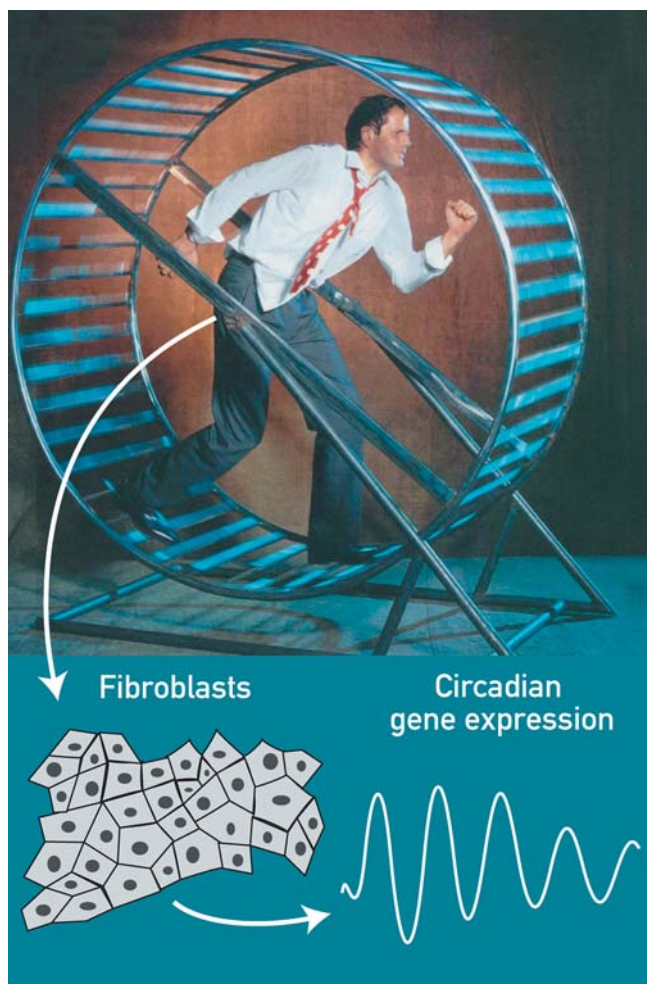
Fibroblast Culture Cells Keep Track of Circadian Rhythms

DOI: 10.1371/journal.pbio.0030348

Embedded in the brain’s hypothalamus on top of the intersection of the two optic nerves lies a group of some 10,000 cells known as the suprachiasmatic nucleus. This neural region determines the biological equivalent of Greenwich Mean Time: the circadian cycle. Circadian rhythms influence phenomena as diverse as leaf position in plants, fatigue patterns in mice, and late-night hunger in human adolescents. By measuring exactly how long an organism takes to complete one cycle, scientists hope to gain insight into the mechanisms underlying these broad effects. “Circadian” translates from Latin to “approximately a day,” but scientists search for more precise answers about how genes and environment account for fluctuations in circadian cycle length.

Theoretically, scientists could measure the circadian signal from its source in the suprachiasmatic nucleus—but no method exists to do this in a living subject. Instead, scientists rely on prolonged observation of live mice or humans. They document when mice use their mouse wheel, for example, and when they sleep. But to screen for and identify circadian rhythm variations in humans, the required period of lengthy observation is prohibitively costly and labor intensive.

Circumventing these technical limitations, Ueli Schibler and colleagues have recently designed a method to measure circadian cycles in mammalian cells cultured from tissues other than the suprachiasmatic nucleus. The researchers infected



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Studying circadian rhythms in humans isn't as straightforward as it is with lab rodents. As a proxy for humans, researchers compared circadian gene expression in primary fibroblasts from different human individuals and found unexpectedly large differences between the circadian clocks of different human subjects.

human and mouse fibroblast tissue cultures with a virus engineered to report when a certain host circadian rhythm gene was expressed. They found that their data jibed with the previously accepted length of the human circadian cycle: 24.5 hours. Because of the sensitivity of their method, Schibler and colleagues also confirmed that, for both humans and mice, circadian rhythms vary substantially between individuals. This suggests that the genetics of the circadian clock likewise varies between individuals.

The so-called reporter gene, luciferase, produces a protein that illuminates the cell. Steve Brown, a postdoc in Schibler's laboratory, created a virus system that specifically inserts luciferase into the host genome near a gene important for establishing the circadian rhythm. With this system, the same gene that promotes expression of the circadian rhythm gene also promotes expression of luciferase—consequently, the fibroblast cultures light up in time with the circadian clock.

Schibler and colleagues obtained human fibroblast skin cells from the abdomen, buttocks, male foreskin, and other areas to measure the circadian rhythm indirectly. Unlike an individual's

true rhythm, a fibroblast cell culture's rhythm does not vary with changes in light exposure or sleep habits. The researchers point out that their method can expose differences in circadian rhythms; it does not, however, directly measure the signal from the suprachiasmatic nucleus that coordinates each individual cell's clock.

The authors also found that circadian rhythm variations between cultures from the same individuals differed far less than differences between individuals. This discovery indicates that fibroblast cell cultures are reliable tools for approximating an individual's genetically determined circadian rhythm. The scientists also found that, for mice, circadian rhythm time obtained from prolonged observation and that obtained from the new viral method were showing the same tendency, although the differences observed in behavior were exacerbated in molecular fibroblast rhythms. This result suggests that the fibroblast circadian clocks might give relevant information about the brain's circadian clock in humans as well as in mice.

In the future, scientists may use the new method to screen large populations for genetically linked sleep disturbances such as advanced and delayed sleep phase syndromes. They may also use this test to home in on the genetic mechanism responsible for such conditions. Outside the realm of medicine, future genetic studies of circadian rhythm may exploit the method developed by Schibler and colleagues to explore questions about the exact relationship between the suprachiasmatic nucleus and the circadian rhythms of cultured fibroblast cells. Just how does this brain structure coordinate genetic imperatives with environmental input including light fluctuations? Finally, frequent fliers, bleary-eyed in foreign time zones, may get an answer to why waking up is so hard to do.
—Jessica Tanenbaum

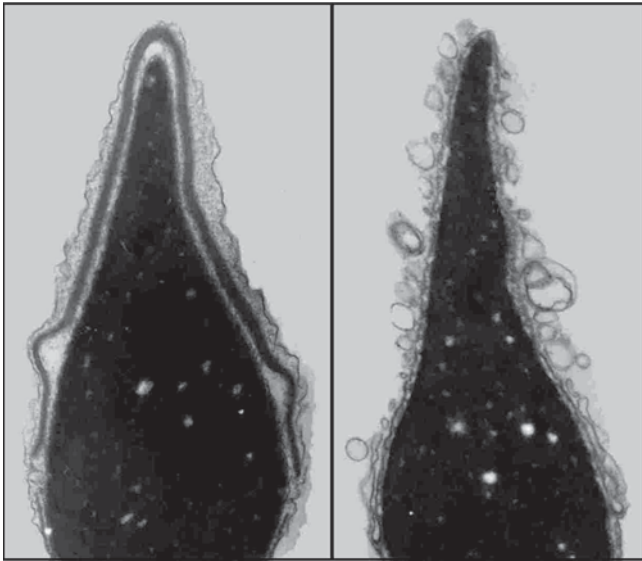
Brown SA, Fleury-Olela F, Nagoshi E, Hauser C, Juge C, et al. (2005) The period length of fibroblast circadian gene expression varies widely among human individuals. DOI: 10.1371/journal.pbio.0030338

EnSNAREd by the Sperm Acrosome

DOI: 10.1371/journal.pbio.0030352

Organelles in cells communicate with each other via small membrane-bound vesicles that carry molecular cargo from one part of the cell to another. Just as a cargo ship must know where to dock in order to empty goods at the correct port, vesicles in a cell must dock and empty their contents in the appropriate part of the cell. Studying the regulation of vesicle transport and membrane fusion is a major area of research in cell biology. Though the rules governing vesicle transport and fusion in the sea of cellular organelles have been deciphered in bits and pieces from various cell systems, all the components required for vesicle fusion have not been characterized for any single cell type. In a new study, Gerardo De Blas, Carlos Roggero, Claudia Tomes, and Luis Mayorga have elucidated the molecular mechanics of membrane fusion by studying the single vesicle of the sperm, the acrosome.

Enzymes released from the acrosome facilitate contact between the sperm and egg membranes during fertilization by dissolving the sheath surrounding an egg. For this, the acrosome membrane must fuse with the outer sperm membrane—a process called the acrosome reaction. This reaction happens only once in the lifetime of a sperm. In other systems, proteins



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Nonstimulated human sperm (left) and sperm undergoing acrosomal exocytosis (right). Multiple fusion events between the cell membrane and the membrane of the acrosome (the sperm's vesicle) trigger release of acrosomal content and formation of membrane-bound vesicles, thanks to the action of SNARE complexes.

involved in membrane fusion must be recycled so they can be reused in the fusion of a subsequent vesicle. But the acrosome reaction is unidirectional and requires no recycling, making it easier to decipher the steps involved.

In previous work, Mayorga's group had shown that an increase in the concentration of intracellular calcium activates a molecule called Rab3A, thus initiating the acrosome reaction. The reaction proceeds with the help of various proteins, including NSF, SNAREs, and synaptotagmin VI, as well as

calcium release from within the acrosome. Synaptotagmin VI is a calcium-sensitive protein. SNAREs are highly specialized proteins that exist in complexes of three molecules wound together in a helix and are present on both the acrosome and plasma membranes. NSF unwinds these helices so that molecules on opposite membranes can interact. To determine whether the SNARE proteins are in a single or triplet configuration at any given time, the authors used bacterial neurotoxins that can degrade single SNAREs but have no effect against the triplets.

In this study, De Blas and colleagues combine fluorescent techniques, a light-sensitive calcium chelator (which depletes all the calcium in the acrosome), and chemicals that inhibit specific steps in the cascade, to decipher whether each reaction occurs before or after the release of calcium. The researchers show that Rab3A activates NSF, which goes on to untwine helical, neurotoxin-resistant SNARE complexes on the acrosomal and sperm membranes, allowing opposing SNAREs to interact. Once SNAREs on opposite membranes form neurotoxin-sensitive loose complexes, calcium is released from within the acrosome. One protein that is required after the release of calcium is synaptotagmin VI. This protein, the authors suggest, could be responsible for the tight zippering of SNAREs on opposite membranes, converting them into toxin-resistant complexes. As the helix between molecules on opposite membranes becomes tighter, the membranes get pulled closer to each other, enabling membrane fusion.

How calcium activates Rab3A or synaptotagmin VI and how these proteins carry out their roles at the molecular level remain to be elucidated. However, this study elegantly demonstrates the cascade of players involved in the acrosomal membrane fusion reaction, from start to finish, in a single cell—something that had not been shown before. —*Supriya Kumar*

De Blas GA, Roggero CM, Tomes CN, Mayorga LS (2005) Dynamics of SNARE assembly and disassembly during sperm acrosomal exocytosis. DOI: 10.1371/journal.pbio.0030323