Synopses of Research Articles

Virus Proteins Prevent Cell Suicide Long Enough to Establish Latent Infection

Little more than a small genome encased in protein, a virus can't reproduce without help from the cell it may ultimately destroy. After attaching to the cell membrane, a virus slips inside the cell, then co-opts its transcription and replication machinery to reproduce. Some viruses, such as the Epstein-Barr virus (EBV), enter a latent stage before they start to reproduce. During latency, the virus expresses its own genes, which help maintain the viral genome until replication begins.

In many viral life cycles, including EBV, reproduction continues until the cell bursts and releases a new crop of viruses. As a defense, cells undergo programmed cell death, or apoptosis, which protects other cells by containing the invader. But just as the host depends on apoptosis to survive, viral survival depends on preventing apoptosis. To do this, many viruses use relatives of a protein called Bcl-2 (the viral version is called vBcl-2). Though many vBcl-2–like proteins, or homologs, can trigger apoptosis, all the viral Bcl-2 homologs identified so far inhibit apoptosis during viral reproduction.

In a new study, Markus Altmann and Wolfgang Hammerschmidt use EBV as a model system to explore whether viral proteins play a role in latent infection. EBV, a tumor-causing herpes virus, targets B lymphocytes, the immune system's antibody-producing cells. While most herpes viruses encode one Bcl-2 gene, EBV encodes two, *BALF1* and *BHRF1*. *BHRF1* is known to prevent apoptosis in cells, but there is no consensus on *BALF1* function.

Altmann and Hammerschmidt created several viral mutants to probe *BHRF1* and *BAFL1* function. Interestingly, they found that blocking the function of both genes had no effect during viral reproduction. But what about the latent stage of the EBV life cycle? To investigate this question, the authors compared *BHRF1* and *BAFL1* activity with that of EBV genes expressed during latency. While 11 latency genes are expressed throughout the latent phase of EBV's life cycle, Altmann and Hammerschmidt found that both *BHRF1* and *BAFL1* act transiently—their transcripts were detected just 24 hours after infection, but not three weeks later, when the other genes were still active.
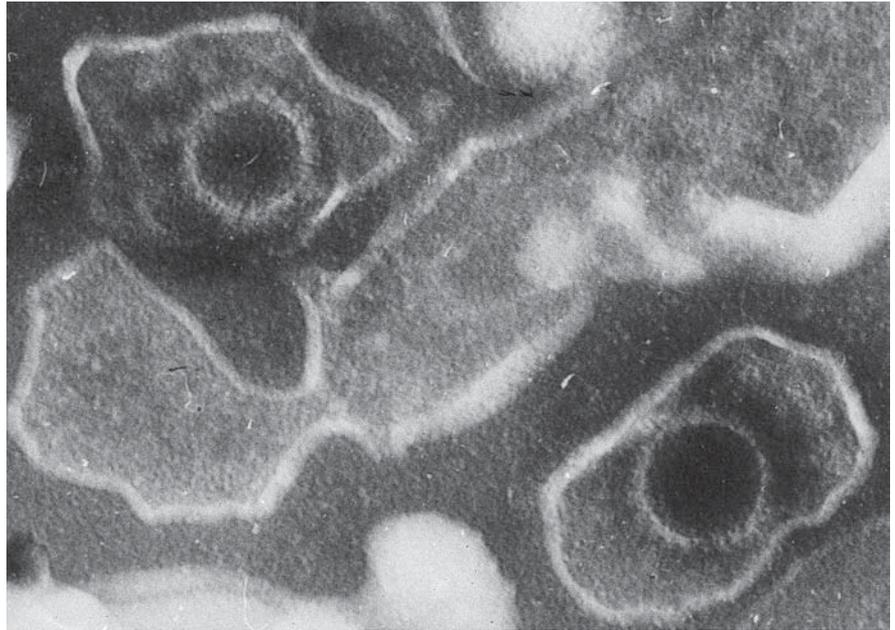
This electron microscopic image of two Epstein Barr Virus virions (viral particles) shows round capsids—protein-encased genetic material—loosely surrounded by the membrane envelope.

This unexpected temporal regulation of gene expression suggested that *BHRF1* and *BAFL1* activity might initially regulate apoptosis in the infected cells. One of the genes linked to latent EBV infection, called *EBNA2*, regulates other latent genes and is required for the establishment of latency. To tease apart the effects of *EBNA2* from those of *BHRF1* and *BAFL1*, the authors infected nondividing primary B lymphocytes with mutant viruses lacking *EBNA2* in one set of experiments, and with double mutants lacking both *BHRF1* and *BAFL1* in another set. Three days after infection, some 70% of cells infected with the double-mutant virus were dying. "In stark contrast," the authors noted, all the B lymphocytes infected with the *EBNA2* mutants—which expressed *BHRF1* and *BAFL1* right after infection—were still alive.

Once the virus is persistent in B lymphocytes, it can transform the cells into a cancerous state called lymphoblastoid cell line. This transformation did not occur when both *BHRF1* and *BAFL1* were nonfunctional. But when the authors reconstructed the double-mutant strain with just one functional gene, the virus regained its ability to transform the cells. Thus, the two genes are redundant—if one is disabled, the other can take its place.

Altogether, these results show that *BHRF1* and *BAFL1* play a critical role in establishing latent infection by preventing apoptosis until the latency genes can be activated. After the virus penetrates into the cell, either *BHRF1* or *BAFL1* keeps the cell alive initially, then *EBNA2* activates the latency genes, allowing the virus to persist and promote B lymphocyte transformation. Because vBcl2 homologs had not been directly linked to latent infection before, this study shines new light on the resources viruses use to bypass the host's defenses. Future studies can begin to explore the mechanisms regulating *BHRF1* and *BAFL1* expression, which may ultimately suggest ways to disrupt their activity and interfere with the virus's success. —*Liza Gross*

Preparing for Transcription: The Role of Histone H2A.Z

Every cell in our body contains the instructions for life encoded in around two meters of DNA. In eukaryotic cells (cells with nuclei), all this DNA is squeezed into the cell's nucleus, a region about one-hundredth of a millimeter across. Cells accomplish this improbable task with the help of histones. These proteins combine with DNA to form chromatin, which is made up of structural units called nucleosomes. Nucleosomes, in turn, consist of about 146 base pairs of DNA wrapped around an eight-unit structure containing two molecules each of four core histones—H2A, H2B, H3, and H4. Each nucleosome is separated from its neighbors by a short "linker" DNA. This "beads-on-a-string" arrangement folds into a smooth fiber, which folds into thicker fibers so that all the DNA packs neatly into the nucleus.

Unfortunately, this tidy solution renders chromatin-packaged DNA mostly inaccessible to the transcription machinery. Consequently, cells have devised several ways to adjust the position and/or characteristics of nucleosomes to allow gene expression, including the incorporation of variant histones into nucleosomes. The evolutionarily conserved histone variant H2A.Z (also called Htz1 in yeast) is implicated in transcriptional regulation and gene silencing (inactive, or silent, genes are packaged into dense chromatin called heterochromatin), but little is known about how H2A.Z, which replaces H2A in some nucleosomes, regulates these biological functions. Knowing exactly where in the genome H2A.Z takes the place of H2A should provide insights into how this particular variant histone regulates genome structure and function—which is why Benoît Guillemette and colleagues set out to map H2A.Z binding sites throughout the yeast genome.

The researchers used a technique called chromatin immunoprecipitation to isolate DNA sequences bound to specific histones in living yeast cells, then amplified them, and determined their position in the yeast genome using microarray analysis. They report that H2A.Z binds to 4,862 small regions (which they call Z loci) scattered across the yeast genome. 74% of these regions lie over promoters, regulatory DNA sequences at the start of transcribed genes; 63% of yeast promoters are decorated with H2A.Z. The authors show that H2A.Z specifically associates with one or two nucleosomes within the promoter of some inactive genes but is generally absent from promoters of highly active genes. In addition, they provide evidence that the chromatin structure is more organized in terms of exact positioning of the nucleosomes and that H2A.Z may play a role in that promoter-specific chromatin organization.

The authors also describe the physical pattern of binding of H2A.Z to a second type of region in the genome—Htz1-activated domains (HZADs). These domains are found in euchromatin (loosely packed, actively transcribed chromatin) lying next to heterochromatin, and it is thought that H2A.Z binding stops the spread of silencing into euchromatin, a function called antisilencing. The researchers report that H2A.Z occupies a wider region around HZAD genes than it does at Z loci, indicating that H2A.Z may affect gene transcription and antisilencing through different mechanisms.

Overall, the authors propose that H2A.Z has two roles in yeast cells: to poise genes for transcription initiation in euchromatin and to protect euchromatin from silencing. Whether H2A.Z incorporation into nucleosomes is necessary and sufficient for these activities, and whether it has additional transcriptional effects in yeast and other organisms, is not yet known, but this map of H2A.Z binding sites in the yeast genome will be invaluable in future investigations into the mechanisms of gene regulation. —*Jane Bradbury*

Guillemette B, Bataille AR, Gévry N, Adam M, Blanchette M, et al. (2005) Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. DOI: 10.1371/journal.pbio.0030384

What Makes Mice Fat? How the Brain Controls Energy Balance

In most animals, food intake and energy expenditure vary greatly from day to day. Yet, in healthy young animals, cumulative energy intake over several days matches energy use very closely. This balancing act or "energy homeostasis" is controlled by complex neuronal circuitry and numerous signaling molecules. When these control mechanisms go wrong, the result is weight loss or obesity. Middle-aged spread, for example, is probably caused by a progressive impairment of energy homeostasis.

Two types of neurons in the hypothalamus—a region deep in the brain that controls many aspects of physiology—help to regulate fat buildup, or adiposity. First, there are proopiomelanocortin (Pomc) neurons, so called because they make

Normal mouse hypothalamic tissue (left) and mutant tissue (right), with Pomc neurons deleted.

proopiomelanocortin. This is a precursor for melanocortins, peptides that bind to melanocortin receptors elsewhere in the brain to limit food intake and increase energy expenditure. Then there are agouti-related protein (Agrp) neurons. These make agouti-related protein, named for its similarity to a

protein mutated in an obese mouse with a characteristic yellow coat. By blocking melanocortin receptors, Agrp increases food uptake—the scientific term for this is hyperphagia—and decreases energy use. Both types of neurons detect circulating indicators of body adiposity such as leptin, and then act to keep energy stores constant.

Support for this model for energy homeostasis comes from rodent studies in which the hypothalamus was damaged or stimulated, or in which leptin and other peptides were injected directly into the brain. Genetic experiments in mice provide further support but also some conflicting evidence. While deletion of the Pomc gene or overexpression of Agrp increase appetite and obesity as predicted by the model, unexpectedly, deletion of the Agrp gene does not disturb energy balance. One explanation for this is that Pomc and Agrp neurons might play a role in energy homeostasis even when they don't express their defining peptides; it is known, for instance, that Pomc and Agrp neurons express additional neuropeptides with effects similar to Pomc and Agrp, and that Agrp

neurons control the activity of Pomc neurons.

To investigate more fully the roles that Pomc and Agrp neurons play in energy homeostasis, Allison Wanting Xu et al. have constructed mouse strains in which the Pomc or Agrp neurons are lost progressively after birth. They took advantage of a technique that selectively deleted the gene for the mitochondrial transcription factor A (Tfam) in Pomc- or Agrp-expressing neuronal cells. Tfam is required for transcription of the mitochondrial genome, which encodes proteins required for cellular respiration and thus cell survival. By six months old, the researchers report, the engineered mice had lost many of their Pomc or Agrp neurons but no other neurons.

Like aging humans, mice in which Pomc neurons had died became progressively fatter because of an increased food intake and reduced energy expenditure. Mice that had lost Agrp neurons weighed slightly less than control animals, and mice engineered so that both types of neurons died weighed more than control mice but less than those lacking just Pomc neurons. These

results indicate that the regulation of adiposity by Pomc and Agrp neurons is not simply a matter of releasing these two neuropeptides.

Xu et al. made an additional, unexpected observation. After food deprivation, mice normally increase their food intake acutely until their fat stores return to prefasting levels—a process called compensatory hyperphagia. Paradoxically, mice without Pomc neurons showed reduced compensatory hyperphagia despite overeating under normal conditions. Since aging humans also fail to increase their food intake after fasting, these mouse strains that gradually lose specific hypothalamic neurons provide a potentially informative model of human age-related obesity. In addition, by studying such mice, scientists may gain important insights into the full complexity of how hypothalamic neurons regulate energy balance that could help to reverse the current human obesity epidemic. —*Jane Bradbury*

Xu AW, Kaelin CB, Morton GJ, Ogimoto K, Stanhope K, et al. (2005) Effects of hypothalamic neurodegeneration on energy balance. DOI: 10.1371/journal. pbio.0030415

Selenium Speeds Reactions

At the heart of every reaction of every cell lies an enzyme, a protein catalyst. At its active site—a special pocket on its surface—it binds reactants (substrates) and rearranges their chemical bonds, before releasing them as useful products. Rearranging some bonds may require help from certain chemical elements that are present in trace amounts. Many enzymes place these elements at the center of their active sites to do the most critical job.

Selenium is one such element. In large quantities, selenium is toxic, but, in trace amounts, it is absolutely essential for life in many organisms, including humans. Selenium is present in proteins in the form of selenocysteine, a rare amino acid that helps promote antioxidant reactions. These selenocysteine-containing proteins are called selenoproteins. One important selenoprotein is the enzyme methionine-*R*-sulfoxide reductase (MsrB) 1, whose job is to repair proteins injured by oxidative damage, caused by sunlight, toxic chemicals, or a variety of other insults.

In mammals, there are two other forms of MsrB, which also can efficiently perform this task, but use the abundant amino acid cysteine instead of selenocysteine. So why do cells go to the trouble and metabolic expense of acquiring selenium from the environment? In this issue, Hwa-Young Kim and Vadim Gladyshev explore the details of active-site chemistry of these three related enzymes, and show that the selenoprotein form employs a different catalytic mechanism.

The authors began by identifying three key amino acids in the active site of the cysteine-containing forms, which did not occur
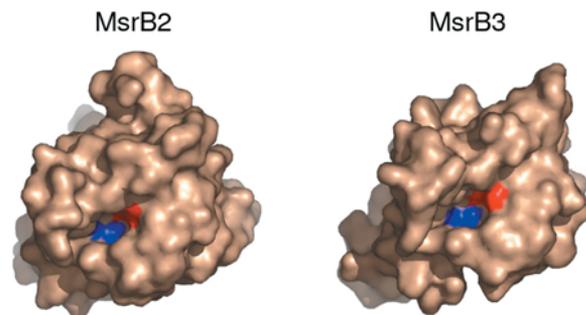
These surface models of mouse MsrB2 and human MsrB3 show the catalytic Cys95 residues in red and the Asn97 residues in blue.

in the selenoprotein MsrB1. When any of these amino acids were mutated, the activity of the cysteine-containing enzymes was greatly diminished. This result indicates that these amino acids likely play a role at the active site, a supposition supported by previous work on related enzymes in bacteria.

Kim and Gladyshev next systematically mutated MsrB1 to include one, two, or all three of these amino acids, and discovered that inclusion of one or any combination of them diminished activity of the selenocysteine-containing enzyme. This suggested that while these amino acids support the mechanism of the cysteine-containing forms, they interfere with the mechanism of the selenoprotein. Not surprisingly, when the selenium was removed from MsrB1, the enzyme was significantly

impaired. But when the three amino acids were added to this crippled enzyme, they restored some of the diminished activity, probably by carrying out the same mechanism they do in the cysteine-containing enzymes.

The authors then inserted a selenium atom into each of the cysteine-containing enzymes, in the same spot in the active site where it sits in MsrB1. They found that the initial activity of each enzyme was increased over 100-fold, indicating the inherent capacity of selenium to promote catalytic activity. These souped-up enzymes were unable to complete the reaction, however, because they lacked other features of MsrB1's active site. Further scrutiny of the enzymes revealed these critical features, and inserting them allowed the artificial selenoproteins to carry out the entire reaction.

The authors suggest the explanation for these findings relates to a difference in the catalytic mechanism of selenocysteine- and cysteine-containing enzymes. The substrate for both enzyme types, methionine-R-sulfoxide, is found within oxidized proteins. The job of both enzymes is to reduce this compound back to the amino acid methionine. Both do so by accepting an oxygen atom from the sulfoxide.

In the presence of selenium, the oxygen temporarily binds to the selenium. The selenium's electrons then shift to bond with a sulfur on a neighboring cysteine amino acid, kicking out the oxygen as part of a water molecule. Finally, the selenium-sulfur bond is broken and the enzyme is restored to its original state by the intervention of thioredoxin, a ubiquitous cell molecule whose job is to undo just such temporary linkages in a wide variety of enzymes.

Without selenium, the oxygen binds directly to sulfur, and thioredoxin intervenes to form the water and restore the sulfur. This reaction occurs in fewer steps, but is slower. The authors propose that the evolution of selenium-containing MsrB1 from cysteine-containing forms was likely favored by the higher rate of reaction it offered, although this trend is likely limited by the requirement for changes in other portions of the enzyme to accommodate the trace element. The authors suggest that selenium provides inherent catalytic advantages to certain types of enzymatic reactions, even though utilization of these advantages is sometimes tricky. If so, manipulation of related enzymes by insertion of selenium may increase their catalytic efficiency, perhaps much above that designed by nature. This may offer advantages for some biotechnology and biomedical applications that depend on antioxidants. —*Richard Robinson*

DNA Barcodes Perform Best with Well-Characterized Taxa

With species around the world disappearing faster than biologists can identify them, the need for rapid, accurate methods of classifying life has never been more pressing. Toward this end, many scientists pinned their hopes on DNA barcoding, a recently proposed strategy that treats a short fragment of DNA as a sort of universal product code to identify species by running unknown sequences through a database that links DNA barcodes to organisms. But this approach generated controversy from the start, with advocates touting the benefits—rapid identification of unknown individuals and discovery of novel species—and skeptics bristling at the notion that a single gene fragment could perform such a tall task.

For most animals, the DNA barcode consists of just over 600 base pairs of a mitochondrial gene called *cytochrome oxidase subunit I (COI)*. In September 2004, *PLoS Biology* published a paper that tested *COI* barcode performance using a proportion of North American birds. The study found that all these well-studied species had a different barcode, and that the variation between species was much higher than variation within species. Based on this gap, the study proposed a screening threshold of sequence difference (ten times the average within-species difference) that could speed the discovery of new animal species. In a new study, Christopher Meyer and Gustav Paulay revisit the issue with a diverse, extensively studied snail group, the ubiquitous, tropical marine cowries whose shell can command over $30,000. Meyer and Paulay found that while the barcode worked well for identifying specimens in highly characterized groups, thresholds would miss many novel species.

After ten years of collecting and sequencing cowries from around the world, Meyer and Paulay assembled a database of over 2,000 cowrie *COI* sequences from 218 species. To capture the full range of within-species variation and geographic differences in population structure, they included sequences from multiple individuals and geographic extremes. Meyer and

The charismatic cowrie.

Paulay tested barcode performance in species identification and discovery against traditional morphology-based species lists and against an integrated taxonomic approach that determines "evolutionary significant units" (ESUs) based on morphology and sequence data. ESUs are what's called reciprocally monophyletic—two ESUs each have a unique ancestor, and, therefore, a unique genetic signature. But genetic variation doesn't always track with species distinctions. The common ancestor of some species nests within another species' variation (called paraphyly), and sometimes different members of what is thought to be one species can be related to another species and not share a most recent common ancestor (called polyphyly).

Meyer and Paulay found that barcodes could accurately identify unknown samples against a well-characterized database using ESUs, but were "prone to error"—with a 20% failure rate—when

traditional species checklists were used, likely reflecting taxonomic problems mentioned above (lumping similar forms that turn out to be distinct species, for example, or erroneously classifying a specimen with an odd morphology as separate species).

When Meyer and Paulay looked at thresholds to delineate species in cowries, they found considerable abundance of young taxa between intra- and intertaxon variation at both ESU and species levels. Within-species variation among cowries was "substantially higher" than that found in two other marine snails, limpets and turbinids, demonstrating the value of comparative analyses in generalizing limits for intra-species variation. The three groups also showed a wide range of interspecies variation. Still, using a barcode threshold to constrain intraspecies variation worked well for ESUs (98% of the taxa had less than 3% variation).

But error rates were substantial when applying thresholds to species discovery because of the abundance of young taxa. For instance, of the 263 ESUs, 16% artificially lumped with another ESU at the 3% threshold; similar patterns were seen in the turbinids and limpets. Because many traditionally recognized cowrie species are not reciprocally monophyletic based on their COI barcode, when Meyer and Paulay replaced cowrie ESUs with recognized species, both intra- and interspecies variation increased, bumping the error rate above 30%.

This comprehensive analysis demonstrates that relying solely on DNA barcodes masks fine-tuned species boundaries not readily captured in DNA sequences without extensive sampling. The barcode performs best in identifying individuals against a well-annotated sequence database—as demonstrated here with ESUs—and the authors argue that the barcoding movement is well-equipped to help in this effort. But barcoding methods for discovering new species need refinement, they argue, and should be developed in collaboration with taxonomists, systematists, and ecologists into a comprehensive taxonomic framework. Once databases are fully annotated with taxonomically evaluated sequences, error rates should go down. With just 1.7 million species described and some 10 million to go, there's a lot of work to be done. —*Liza Gross*

Meyer CP, Paulay G (2005) DNA barcoding: Error rates based on comprehensive sampling. DOI: 10.1371/journal.pbio.0030422

An Insecure Role for Securin in Chromosome Segregation

Just as good parents try to prevent inequities among their progeny, a dividing cell must ensure that its daughters inherit all the chromosomes they are entitled to. But with cells, such evenhandedness goes beyond matters of equity to those of life and death. The progeny of cancer cells, for example, typically suffer rampant chromosome losses. Though these losses should eventually cause the cells' demise, they can also reveal or induce mutations that encourage proliferation, which explains why cancer cells accommodate widespread chromosomal instability. Understanding how normal cells unerringly transmit a full chromosome set to their daughters has long been an important part of the fight against cancer.

In the ballet of cell division, or mitosis, chromosomes, intracellular fibers, and the cell outer membrane execute carefully choreographed steps and partner shifts. First, cells replicate their DNA, creating twin sets of each chromosome, known as sister chromatids. Mitosis, per se, starts when the chromatids condense their DNA into compact bodies that are captured in a spindle of tubulin fibers (called microtubules). The microtubules first line up the chromatid pairs along the spindle's middle plane; then they pull them apart, hauling the members of each pair to opposite ends of the spindle. The mother cell then pinches its membrane along the spindle's middle plane, splitting into two daughter cells with full chromosome sets. Keeping the chromatids of a pair together at the beginning of mitosis, and allowing their timely separation at the end, are both crucial steps for proper chromosome segregation into the daughter cells.

From the time DNA replication begins, sister chromatids are held together by a protein complex named cohesin. After the chromatid pairs are all neatly positioned in the center of the spindle, they can be safely segregated, a job performed by separase, an enzyme that dissolves cohesin's grip by breaking down one of its protein components. The dissolving power of separase is tightly controlled to avoid precocious or delayed chromatid separation. One of separase's regulators is securin, a protein known as a chaperone that appears to hold separase captive until just the right time. A recent study indicated that human cells devoid of securin underwent abnormal mitoses that led to widespread chromosome losses, making securin a key player in chromosome segregation and a promising entry point into cancer therapy. But in a new study in *PLoS Biology*, Katrin Pfleghaar, Michael Speicher, and colleagues have repeated and expanded on these experiments, and come to somewhat different conclusions.

Both teams carried out the same experiments in the same system: they counted chromosomes and examined the mitotic process in cultures of human cells lacking the securin gene. And both teams found that in the first weeks of cell culture, cells were losing chromosomes at a very high rate and most mitoses showed abnormal chromatid distribution. But in this study, Pfleghaar observed the cells for longer periods and found that as time went by, the culture recovered: cells with abnormal mitoses and chromosome counts became rarer until, after a few weeks, the cells appeared indistinguishable from their relatives with an intact securin gene. Interestingly, both studies found that the amounts and activity levels of separase were low in securin-deficient cells, which confirms that securin regulates separase. Pfleghaar and her colleagues speculate that securin normally plays an important role in mitoses, but that in its absence, cells tap into compensatory mechanisms to restore proper chromosome segregation.

The implications for cancer treatment are potentially great, as mathematical models of cancer growth do not usually include the possibility that cell populations might recover from chromosomal instability. In addition, such recoveries might interfere with therapies that aim to kill cancer cells by exacerbating their chromosome losses. —*Francoise Chanut*

Pfleghaar K, Heubes S, Cox J, Stemmann O, Speicher MR (2005) Securin is not required for chromosomal stability in human cells. DOI: 10.1371/journal.pbio.0030416

A Systematic Way to Find Linear Motifs Mediating Protein–Protein Interactions

If John Donne had been a systems biologist, instead of writing "No man is an island," he might have written "No protein is an island." Proteins in cells interact with numerous partners, forming complex networks that keep cells ticking over, allow them to respond to external stimuli, and make sure that essential processes like cell division go smoothly.

Interacting proteins can be identified experimentally in many ways. For example, an antibody recognizing one protein can be used to pull that protein plus any proteins that are bound to it out of cell extracts. High-throughput assays have also been developed that can screen whole genomes for genes encoding interacting proteins. But we remain a long way from having an accurate picture of all the protein–protein interactions in even one cell, let alone a whole organism.

Proteins interact with each other in two main ways. The first is through globular domains, which are formed when linear strings of 100–200 amino acids fold into specific shapes determined by their amino acid sequence. The second is through a globular domain in one protein and a short linear sequence (motif) of three to eight amino acids in the other. The structures of globular domains are relatively easy to solve, and how they interact in well-established pairs of interacting proteins can be visualized. Furthermore, other potentially interacting domains can then be inferred through sequence similarity to known globular domains. By contrast, the linear motifs involved in protein–protein interactions can't be easily identified by sequence comparisons. Until now, the only way to find them has been through time-consuming experiments, and consequently, only a few hundred linear motifs are known.

Now, a systematic way to find linear motifs in the billions of sequences stored in databases, devised and tested by Victor Neduva et al., ushers in a new era in understanding protein–protein interaction networks. The researchers started with the hypothesis that a set of proteins with a common interacting partner will share a feature that mediates binding. This feature could be a domain or a linear motif. To find the latter, they stripped away the sequences of globular domains and of long repetitive regions to leave behind the nonstructured parts of the proteins, regions where they believe linear motifs are most likely to lie. They then determined whether any short sequences in these protein remnants occurred more frequently within the set of interacting proteins than would be expected by chance. These statistically significant short sequences are potential linear motifs involved in protein binding.

The researchers tested their approach on protein sets sharing known motifs, and showed that it efficiently identified these motifs while minimizing the number of false positives. They then extracted protein sets that shared a common interaction partner from four species-specific datasets of protein–protein interactions and ran their protocol for finding linear motifs. From a fly dataset, for example, they identified 26 protein sets with one or more linear motifs that occurred more frequently than expected by chance. That nine of these motifs were already known provided important validation of their approach, but the researchers also checked several of the new motifs in direct binding experiments. For example, they showed that a motif predicted to bind to the fly protein Translin did in fact bind to it; a mutated version of the motif did not.

From their results, Neduva et al. estimate that hundreds of linear motifs may remain to be discovered. Given the central role that linear motifs play in protein–protein interactions, their systematic method for finding them should rapidly improve our understanding of the complex network of protein–protein interactions that drives the everyday lives of cells. —*Jane Bradbury*

Neduva V, Linding R, Su-Angrand I, Stark A, de Masi F, et al. (2005) Systematic discovery of new recognition peptides mediating protein interaction networks. DOI: 10.1371/journal.pbio.0030405

A Surprising New Path to Tumor Development

In the long-established model of tumorigenesis, cells acquire specific mutations that disrupt the normal regulatory constraints on unlimited cell division. These mutations allow cells to proliferate at the expense of healthy tissues and develop into tumors. As a result, most cancer treatments use agents that curb tumor growth by inhibiting cell divisions. Surprisingly, a few conditions known to reduce a cell's ability to divide—including genetic traits, some diets, and chemotherapies—seem to increase the chances of tumor occurrence. In a new study, Ganna Bilousova and her colleagues shed new light on this troubling paradox by examining the development of mouse blood cell tumors (leukemias) in contexts that reduce cell division rates.

All circulating blood cells are descendents of progenitor cells that reside in the bone marrow. The ultimate progenitors, called hematopoietic stem cells, give rise to all blood cells by producing more specialized progenitors that initiate distinct lineages, such as the red and white blood cells. Progenitors divide to ensure the constant renewal of blood cells, but their cell divisions must be tightly controlled to avoid generating too many blood cells and increasing the risk of tumors.

Overproliferation of blood cell progenitors seems to underlie chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). The blood progenitors of both leukemias harbor a genetic anomaly known as the Philadelphia chromosome. This aberrant joining of Chromosomes 9 and 22 produces an aberrant protein called Bcr-abl, which promotes both cell proliferation and mutations. Either property could account for Bcr-abl's cancer-promoting, or oncogenic, potential. Yet, as the authors show, simply introducing a few blood progenitors carrying the Bcr-abl protein is not enough to produce leukemias in mice. Only when cell divisions are impaired in the mice blood progenitors does the proliferative advantage, and oncogenic potential, of Bcr-abl become obvious.

Their experimental setup mimics the conditions of leukemia onset—in which an oncogenic mutation presumably arises in a single (or a few) blood progenitors—yet eventually allows
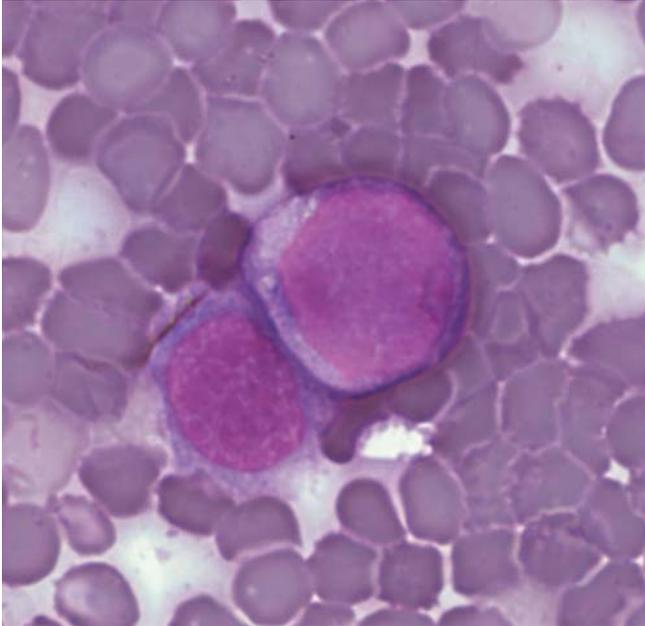
DOI: 10.1371/journal.pbio.0030433.g001

Leukemia cells.

the cell to supersede its nonmutant counterparts. The authors transplant a mixture of two types of progenitors—some express Bcr-abl and some don't—into mice, and examine their relative contribution to the recipients' blood a few weeks later. (The rodents' own blood progenitors had been previously destroyed

by irradiation.) The authors find that whether they slow cell divisions genetically (using stem cells with mutations in key regulators of cell divisions) or chemically (feeding transplanted mice hydroxyurea, a drug commonly used in cancer therapy), the progenitor cells that carry Bcr-abl can overcome the hurdle and produce mature blood cells and, eventually, tumors. In the absence of this challenge, however, Bcr-abl cells barely contribute to the recipients' blood, and far fewer cancers arise. What's more, the authors can quench the oncogenicity of Bcr-abl progenitor cells simply by cotransplanting normal progenitor cells (free of Bcr-abl and able to divide).

The implication of these observations is that nonmutant progenitor cells normally outcompete a Bcr-abl cell arising in a bone marrow niche. But under conditions that impair cell divisions, whether genetic, environmental, or therapeutic, the mutated cell has the advantage and might eventually take over. Overcoming cell division blockers is a tall order that not all oncogenes are expected to fill. Yet mutations in *p53*, an oncogene linked to a wide variety of cancers, confer the same advantage as Bcr-abl in this experimental setting, which suggests that the authors' model might apply to other tumors as well. While much more work needs to be done for the implications of this study to affect people, Bilousova and colleagues speculate that preventing cancer not only involves avoiding exposure to mutation-causing substances, but also a lifestyle that promotes healthy cell division behavior among tissue progenitors.

—*Francoise Chanut*

Bilousova G, Marusyk A, Porter CC, Cardiff RD, DeGregori J (2005) Impaired DNA replication within progenitor cell pools promotes leukemogenesis. DOI: 10.1371/journal.pbio.0030401

Selection on a Neural Gene Regulator Sheds Light on Human Evolution

DOI: 10.1371/journal.pbio.0030417

With humans and chimpanzees differing by just 1.2% at the DNA level, it's clear that our differences do not arise from gene variation alone. Thirty years ago, Mary-Claire King and Alan Wilson pointed to our extensive protein similarities as evidence that those investigating the genetic basis of human origins should focus on the regulators of gene expression rather than on the genes themselves.

DNA sequences that regulate gene expression, called *cis*-regulatory elements, occur on the same DNA molecule as the regulated gene. By recruiting proteins that initiate or block transcription, *cis*-regulatory elements influence the rate at which genes are transcribed, in which cells, and under what conditions. But since sequence inspection doesn't reveal whether regulatory sequence changes are functional or neutral, finding evidence for human-specific changes and positive selection in these sequences is far harder than finding similar evidence in protein sequences or in gene sets. Now, Matthew Rockman, Gregory Wray, and

their colleagues provide further support for King and Wilson's predictions by showing that positive selection altered the *cis*-regulation of a gene expressed in the human brain.

To find evidence of regulatory changes underlying uniquely human traits, Rockman et al. examined the regulatory evolution of *prodynorphin*, a gene expressed in multiple brain and endocrine cell types. The protein encoded by *prodynorphin* is a precursor molecule for a suite of neuropeptides that bind to opiate receptors and affect perception, pain sensation, emotion, and learning. In humans, *prodynorphin*'s promoter contains what's called a 68 base-pair tandem repeat polymorphism—individuals can have up to four copies of the 68 DNA base-pair element, which occur side by side. The polymorphism, which affects how many transcripts of the gene are produced, has been tentatively linked to schizophrenia, cocaine addiction, and epilepsy.

To explore how this functional variation evolved, Rockman et al. first

DOI: 10.1371/journal.pbio.0030417.g001

Positive natural selection altered the regulation of the human prodynorphin gene, which produces a product that interacts with the same receptors that opium targets.

sequenced and analyzed *prodynorphin* regulatory DNA from 74 human chromosomes and 32 nonhuman primate chromosomes (chimp, bonobo, gorilla, orangutan, baboon, and two macaque species). The duplication leading to tandem repeats appears

unique to humans, since all the monkeys and other great apes carry only one copy of the 68 base-pair element. Further distinguishing humans from the last common ancestor of humans and chimps, the human copies also carry five mutations, or substitutions, far more than would be expected if the mutations were neutral (that is, had no effect on fitness). Three nearby polymorphisms also occurred at a higher-than-expected frequency in humans, a sign that selection acted on the linked neighboring sequences. The protein-coding sequence of *prodynorphin*, on the other hand, appears to have undergone negative selection, discarding harmful mutations that would disrupt its function.

To determine the functional effects of the human substitution, the authors attached a bioluminescent enzyme to human and chimp *prodynorphin cis*-regulatory DNA, and introduced the modified DNA into human cell lines so they could measure transcription levels. Only the human 68 base-pair element significantly increased transcription of *prodynorphin*, and this increase was seen only in brain cells.

Because different numbers of repeats are associated with different effects, such as protection against cocaine addiction and neurological disease, Rockman et al. searched for signs of recent selection. If selection had occurred, divergence among populations should be increased and variation within populations reduced, relative to the neutral case. And that's what the authors found. The frequency of three-repeat versions of the 68 base-pair element was less than 10% in Chinese and Papua New Guinean populations and over 60% in Italy and Ethiopia. And variation at genome markers called microsatellites was significantly low overall across most of the populations studied (which also included India). Because microsatellites typically undergo high mutation rates, if a microsatellite is linked to an element under positive selection, it should show reduced variation.

The observed pattern of variations within and among human populations, the authors argue, suggests that recent selection has favored different versions of *prodynorphin*-regulatory elements in different regions of the world. These results support the longstanding notion that changes in gene regulation had major impacts on the evolution of novel traits, and may well hold the key to that eternal question, what makes us human?
—*Liza Gross*

Rockman MV, Hahn MW, Soranzo N, Zimprich F, Goldstein DB, et al. (2005) Ancient and recent positive selection transformed opioid *cis*-regulation in humans. DOI: 10.1371/journal.pbio.0030387

Neural Basis of Body Image: How to Lose Inches at the (Perceived) Flick of the Wrist

Wouldn't it be nice if you could change your body image by placing a vibrating gadget on your wrist? As it happens, you can—though under controlled circumstances. Vibrating skin over the tendon of a joint extensor muscle triggers the vivid sensation that the joint is passively flexing, even though it's not. When the hand is touching the waist, nose, or some other body part, a person can feel the wrist bending and the body part stretching or shrinking—in what's aptly called the Pinocchio illusion.

Vibrations on the skin over a muscle tendon cause the perceptual illusion by exciting sensory nerve endings in the tendon that send signals to brain areas that process touch and motor control, the primary somatosensory cortex and the primary motor cortex. The somatosensory cortex creates neural maps of the body surface, and receives sensory inputs from receptors in the peripheral nervous system. But these peripheral receptors carry no information about the relative size of body parts, and the brain has no specialized neurons to receive such information. The neural map of body size and shape are likely represented in a relative way by integrating signals from the relevant body parts and visual cues. The parietal lobes may play a role, based on reports that patients with parietal cortex injuries imagine changes in the size and shape of various body parts. Still,
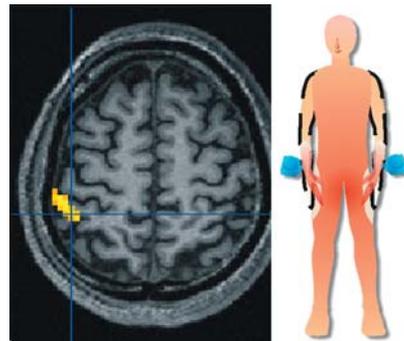
Higher-order somatosensory areas in the parietal cortex mediate perceived changes in body shape and size.

it's not clear how the brain integrates the relevant information to compute body image.

To investigate the neural correlates of body image, H. Henrik Ehrsson, Eiichi Naito, and their colleagues recruited 24 participants to model the "waist shrinking illusion," and then scanned their brains with functional magnetic resonance imaging (fMRI). The authors hypothesized that higher-order somatosensory areas in the parietal cortex would reflect the perceived changes in waist size, and designed the study to isolate illusion-linked brain activity by varying participants' hand position (body contact/no contact, or free) and the vibration site (tendon/skin, or beside the tendon).

After participants experienced each possible combination of hand position and vibration site, they answered "now" when they felt the illusion, and then chose a picture from six different body configurations that best represented their experience. They rated the vividness of the experience, on a scale of zero to nine (absolutely realistic), and then moved their wrists to show what they felt so the authors could measure the angle. At the same time, electromyograms (EMGs) recorded muscle stimulation.

Seven participants did not reliably experience the illusion and so were not scanned. The other 17 participants underwent six experimental trials (two baselines, with hands resting, were added) while their brains were scanned (while lying in the fMRI machine). In three trials, participants' hands lay freely, but supported, by their side without touching the body (tendon free/skin free/rest free). In the other three trials, the palms of the hand were in direct contact with their sides (tendon contact/skin contact/skin free), while a strap allowed them to relax their arms.

During the tendon contact condition, all 17 participants sensed their hands flexing and their waist shrinking. The degree of flexion corresponded to a 28% waist shrinkage. This sensation was

vivid, reliable, and quick to start. The EMGs showed no muscle activity in over 70% of the participants, and muscle activity wasn't significantly different in tendon contact and tendon free, confirming that muscle stimulation did not account for the illusion. The brain regions showing most activity during the illusory perception were in the left parietal lobe, within the anterior intraparietal sulcus (a sulcus is an inward fold of the brain) and extending toward the postcentral sulcus.

Participants who reported the strongest shrinking waist illusion also showed the strongest activity in the postcentral sulcus and the anterior left intraparietal cortex—activity that was not observed in participants who felt illusory wrist movement when their hands were not touching their body—confirming a link between these brain regions and the shrinking waist illusion. When the brain receives conflicting sensory information from the vibrated wrists and the sensory inputs of the hands on the waist, the brain recalibrates the relative size of the wrist and shape of the waist, creating the illusion that the waist is shrinking as the hands are bending inward. Altogether, these results suggest that the brain computes body image by integrating signals from the skin, joints, and muscles through hierarchical processing in the somatosensory system. The researchers could elicit this illusion as many times as needed for the fMRI experiment, but there's no indication that a portable device will hit the consumer market anytime soon. —*Liza Gross*

For Some Genes, Acetylation/Deacetylation Cycling Is the Real Turn-On

The human genome contains some 20,000–25,000 protein-coding genes, but at any given moment, only a small fraction of them is actively transcribed. The DNA that constitutes a gene is wound around multiple nucleosomes, barrel-shaped protein clusters that serve to organize and protect the DNA. When nucleosomes are packed tightly together, the transcription machinery can't easily reach the gene's promoter segment, where it must bind to begin the transcription process; thus, the gene remains silent. Each nucleosome is a bundle of proteins called histones, with ends (tails) that extend from the nucleosome and are accessible for regulatory modification. Modifications can serve two functions. They may regulate how tightly or loosely nucleosomes pack together, or alternatively, they may function as recognition motifs, allowing other regulatory proteins to be recruited to these nucleosomes. In recent years, therefore, histone modifications have come to be appreciated as a major route for controlling gene expression.

One such modification is the addition of a two-carbon acetyl group to histone H3. Histone acetylation has been widely believed to enhance gene expression. But in this issue, Catherine Hazzalin and Louis Mahadevan show that, for at least some genes, dynamic turnover of acetyl groups on the histone, rather than stable acetylation, is the key to turning on the gene.

Acetyl groups are added to histone H3 by acetyltransferase enzymes, and removed by deacetylase enzymes. The authors showed that when they added a deacetylase inhibitor to cultures of mouse cells, the acetylation level of H3 increased, as they expected. But unexpectedly, they found two modes of sensitivity to deacetylase inhibitors. The majority of H3 was largely insensitive to the presence of the inhibitor. In contrast, the minute fraction of H3 already modified by methyl groups at the fourth amino acid in the tail was immediately and very highly sensitive to deacetylase inhibitors, and picked up new acetyl groups rapidly. Methyl modification at position 4 has previously been associated with increased gene activity.

Acetylation was rapid in genes with multiple methyl groups at position 4, such as c-*fos* and c-*jun*. In contrast, the deacetylase inhibitor did not increase acetylation in β-*globin*, which lacks histone H3 methylated at position 4. For those genes in which acetylation increased, not every nucleosome across the entire gene was equally acetylated, and the pattern of increase appeared to be gene specific. Both c-*fos* and c-*jun* had increased histone H3 acetylation at sites adjacent to the gene's promoter and across the start-of-transcription site, but other regions of each gene were affected differentially between the two.

The authors also showed that three different modifications—methylation at position 4, acetylation at position 9, and addition of a phosphate at position 10—can all occur on the same histone H3 molecule. This tight cluster of modifications is likely to induce significant structural changes in this portion of the molecule, setting the stage for further effects associated with increased gene expression. Another recent paper from this group and others describes the function of some of these modifications at these genes, which is to transiently recruit the phosphate-binding adapter protein 14-3-3 to these nucleosomes.

Finally, the authors asked whether the increase in acetylation brought on by deacetylase inhibition led to an increase in gene activity, in keeping with the prevailing model of gene regulation. The transcription of c-*fos* and c-*jun* can be stimulated by the addition of a chemical inducer. But when cells received the deacetylase inhibitor before, or even up to ten minutes after, inducer treatment, both genes were inhibited. This was due to a direct inhibition of the transcription process, and not from effects on cell signaling or other secondary pathways. Thus, it is turnover, or cycling, of acetylation and deacetylation that is needed to increase expression of these genes.

The mechanism by which continuous acetylation/deacetylation cycling promotes gene expression remains unknown, but these findings add to the complex picture of gene regulation that has emerged since the discovery of histone modification. The hypothesis that there might be a "histone code"—a predictable pattern of modifications invariably associated with increased gene activity—appears to be a simplification, and one that does not, at least in some cases, correspond to the actual dynamic system the cell uses to regulate its genes. —*Richard Robinson*

Ankyrin-B Binds Disparate Proteins to Keep Calcium Flowing in the Heart

Muscle cells of the heart regulate their calcium levels tightly, and no wonder—an influx of calcium triggers contraction of the cell, and the beating of the heart. After it enters, calcium must be quickly pumped back across the cell membrane, a job that falls to the sodium/calcium exchanger (NCX1), which trades incoming Na^+ ions for outgoing Ca^{2+} ions. The sodium, in turn, is pumped out by the workhorse of membrane gradients, Na/K ATPase.

Defects in calcium equilibrium, or homeostasis, underlie major diseases of the heart, including arrhythmia, an inability to regulate the heartbeat. One cause of arrhythmia is a mutation leading to loss of the protein ankyrin-B. This mutation increases calcium within heart muscle cells. In this issue, Peter Mohler, Vann Bennett, and colleagues show that ankyrin-B binds to both NCX1 and Na/K ATPase, as well as to a third protein; that mutations in ankyrin-B disrupt this complex; and that the loss of this complex is the likely reason for arrhythmia from ankyrin-B mutation.

Ankyrin-B was known to bind individually to both proteins, as well as a third one, the inositol 1,4,5-trisphosphate receptor ($InsP_3R$). To determine if the entire group formed a single complex, the authors stained the various proteins, and using three-dimensional microscopy, showed that the staining pattern for each largely overlapped. They next used antibodies to precipitate each of the four proteins in turn. They found that, in each case, precipitation of one protein brought the others along with it, strongly suggesting the four formed a single multiprotein complex. This conclusion was further strengthened when they found that the purified proteins created in vitro could also link together. Microscopy revealed that this complex was embedded in an invagination of the plasma membrane called the transverse tubule, or T-tubule. The T-tubule also holds the proteins that allow calcium into the cell, but the staining pattern showed that these were located apart from the ankyrin-B complexes.

Finally, the authors examined how well mutant ankyrin-B binds to the other proteins in the complex. They found that the mutant lost 60% of its ability to bind the other three proteins. Since the physiological effect of the mutation is loss of calcium regulation in heart cells, these results strongly suggest that binding to ankyrin-B is critical for efficiently coordinating the function of the sodium/calcium exchanger with that of the Na/K ATPase, to remove calcium from the cell. The authors note that their results do not explain the function of the $InsP_3R$ protein, whose role in the heart is currently unknown. Earlier evidence suggested it may cooperate with other proteins to regulate calcium influx, but that seems less likely now, given its localization on this complex.

Along with explaining the mechanism of a known defect in cardiac calcium
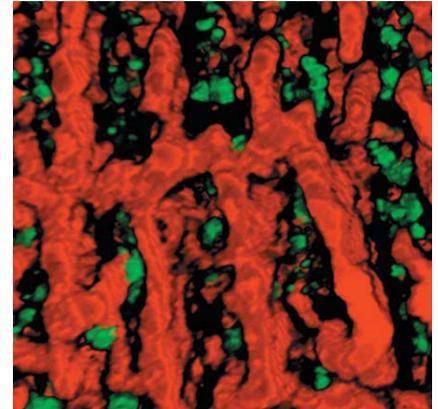
The localization pattern of ankyrin-B (red) and dihydropyridine receptor (green) in cardiomyocytes.

regulation, these results also highlight the important role played by "adapter" proteins such as ankyrin-B in creating "molecular machines." Such multiprotein complexes are common in the cell, and their working depends on the close proximity of member subunits. By bringing together subunits of disparate structures but related functions, such adapter proteins increase the efficiency of the unit as a whole. —*Richard Robinson*

Go West, Early Man: Modeling the Origin and Spread of Early Agriculture

After the last major ice age some 10,000 years ago, things began to look up for early humans. Forbidding climes yielded to more hospitable weather patterns, and people began to settle down and domesticate plants and animals. Archeologist Gordon Childe, who in 1942 called the transition from hunting and gathering to agriculture the Neolithic Revolution, proposed that unchecked population growth triggered economic and social problems among Near Eastern populations and forced farmers and shepherds to search for new lands. In this demic diffusion model, dispersing populations introduced Europeans to the Neolithic lifestyle. Alternately, Europeans may have learned to farm by imitating Neolithic practitioners they encountered through trade or other interactions (the cultural diffusion model).

Childe's ideas of westward migration found support in a 1965 study that mapped the spatiotemporal pattern of a small sample of radiocarbon dates (determined from animal bones and other carbon remains) from Neolithic sites. A landmark study by Albert Ammerman and Luigi Cavalli-Sforza in 1971 used more data—radiocarbon dates from 53 early Neolithic sites—and used a population biology model to investigate Neolithic spread. Their "wave of advance" model proposed that population growth at the agricultural fringes coupled with local migrations would produce steady population expansions in all directions. They calculated an average rate of spread of about one kilometer per year.

But the controversy between the cultural and demic diffusion models still remains today. Now, over 30 years later, Ron Pinhasi and Joaquim Fort revisited the question along with Ammerman, using a substantially larger dataset with new locations— radiocarbon-dated bones and charcoal from 735 Neolithic sites in Europe, the Near East, and Asia—and reaffirm the wave-of-advance model. The authors combined mathematical and geospatial techniques to estimate the timing and likely center of agricultural origins, as well as the rate of spread. Their results support a model of demic diffusion and, for the first time, pinpoint the geographic origin of agriculture within the Fertile Crescent.

Pinhasi et al. calculated the correlation between the straight distance versus age of the 735 radiocarbon dates and the likely spread from 25 hypothetical centers of origin (based on location only) and ten probable centers (sites that included the oldest remains, as well as a center proposed in the 1971 study). The most southern point, Abu Madi in Egypt, had the highest correlation, though eight of the other probable centers had similar scores. However, charting the shortest paths (which take into account the barrier effect of the Mediterranean Sea), pointed to an origin in the north. Focusing on the centers that seemed most likely, Pinhasi et al. used both approaches (one based on straight paths, one based on shortest paths) to estimate the speed of agricultural spread, and came up with nearly the same figure: 0.7–1.1 kilometers per year versus 0.8–1.3 kilometers per year. An error range for this speed was estimated (which had not been done before), so the authors could also compare this observed rate with that predicted by a model.

While no cultural diffusion model is known so far that can explain the observed rate (calculated from the archeological evidence), a kilometer or so a year is consistent with a time-delayed demic diffusion model. (This model, which was proposed by Fort and co-workers in 1999, also agrees with data from other human and nonhuman population expansions, as well as with the observed speeds of virus infections.) While many genetic studies also support demic diffusion, they do not agree on the extent to which Near Eastern farmers contributed to the European gene pool. Assuming a linear advance, agricultural expansion began some 9,000–11,500 years ago, falling in line with a gradual wave of advance. Rather than "racing across the map of Europe," the authors argue, the Neolithic transition took over 3,000 years, or 100 generations, reflecting the time children stay with their parents before moving on to greener pastures. This is precisely the time-delay effect that classical diffusion models are unable to capture, but that is accounted for in the model by Fort and co-workers. Finally, the authors incorporated radiocarbon data from 30 sites in Arabia to find the most likely birthplace of agriculture. Their shortest-path analysis points to northern Levant and northern Mesopotamia (whereas the straight-path, or classical, approach pointed to a southern origin).

The authors' approach did not address whether migrants traveled by land or by sea or whether farmers displaced foragers. But the pattern and processes of dispersal were likely complex, Pinhasi et al. conclude, with multiple paths and mechanisms fueling the western expansion of the Neolithic lifestyle. And with a newly bolstered wave-of-advance model and the approach outlined here, geneticists, anthropologists, and other researchers investigating the origin and spread of human populations have a more detailed roadmap to follow.

Note for international readers: During the time of Westward expansion in the 19th century, American essayist Horace Greeley famously advocated Manifest Destiny by exhorting, "Go West, young man!" —Liza Gross

Music to Her Ears? Male Mice Sing an Ultrasonic Tune

Readers of a certain age may remember Mighty Mouse singing, "Here I come to save the day!" as he raced in to save the innocent from evildoers. Fanciful as his cartoon antics may have seemed at the time (it first aired in the 1950s), the TV show's creators weren't so off the mark. There's still no evidence of superhero behavior in real mice, but in a new study, Timothy E. Holy and Zhongsheng Guo show that mice have a gift for song.

Much of what we know about the biology of song and song learning comes from research on songbirds, but birds are difficult subjects for genetic studies. Song commonly figures in courtship rituals among birds, insects, and frogs, but such behavior in mammals had been restricted to whales, bats, and humans. Evidence of similar behavior in the mouse—a long-established genetic model, often referred to as the pocket human—could open whole new avenues of research into the genetic contributions to song and song learning.

To a lay person, the vocal stylings of rodents appear restricted to squeaks and chatter. But mouse social encounters prompt many vocalizations; some are audible to humans, such as the distress

Male mice serenade potential partners with ultrasonic song.

calls of pups, and some aren't, such as the ultrasonic calls of males presented with females or urine pheromones. Previous studies characterized the situations that prompt these vocalizations, but did not detail their acoustics. In this study, Holy and Guo went beyond the conditions that prompt rodent discourse to focus on the sounds themselves. Far from random patter, male ultrasonic calls contain complex passages with long sequences composed of diverse syllable types.

The authors used cotton swabs coated with either female mouse urine, male mouse urine, or a combination of the two to elicit the male mouse's ultrasonic sounds, and then recorded their vocal responses. The authors manipulated the recordings to hear the ultrasonics. One approach used a slow playback (at one-sixteenth of the recorded speed), but this distorted the temporal structure of the sounds, and the calls sounded like low, intermittent whistles. The other dropped the pitch to an audible level without interfering with the time sequence— the pitch-shifted recording sounds remarkably like birdsong. (To listen, go to DOI: 10.1371/journal.pbio.0030386. sa004.) To bolster this subjective conclusion, the authors then undertook a quantitative analysis of the sounds.

The males produced rapid "chirp-like" syllables of varying duration, spaced at about ten syllables per second, with a burst of closely spaced syllables followed by periods of silence. Some of the syllables showed sudden, significant changes in frequency (or pitch)—all in keeping with previous reports. To determine whether these frequency jumps, or pitch changes, followed a stereotyped pattern or occurred randomly, the authors first analyzed a set of 750 syllables produced by one mouse in a single 210-second trial. They identified discrete clusters of pitch changes, including two clusters with stereotyped jumps to or from a low frequency, which they called low jumps. Repeating the trial and analysis with 45 different mice produced similar results, indicating that the pitch changes are a universal feature of mouse ultrasonic vocalizations.

These pitch jumps formed three distinct categories, which together with two other techniques—borrowed from previous research in birdsong, speech, and pattern recognition—confirmed that syllables are naturally grouped by their pitch changes. The syllables, in turn, occurred in complex sequences that, in some cases, constituted regularly repeated motifs. Syllables with low jumps were repeated in blocks, and phrases tended to be introduced with syllables containing no pitch jumps at all. Since the mice produced multiple syllable types arranged in regular, repeated time signatures, their vocalizations meet the definition of song. Finally, the authors showed that individual males produced songs distinct from those of other males. "The richness and diversity of mouse song appear to approach that of many songbirds," Holy and Guo write. And just like songbirds, the mice appear to be singing their own tune. Future studies can begin to unravel the physiological basis and mechanics of ultrasonic mouse song—and perhaps decipher the messages encoded in the notes and melody. —*Liza Gross*

Holy TE, Guo Z (2005) Ultrasonic songs of male mice. DOI: 10.1371/journal. pbio.0030386

Synchronized Brain Interactions Associated with Memory and Decision-Making

Next time you lose your keys, you might consider the Clark's nutcracker. During the fall, this woodland resident collects over 30,000 seeds, buries them in discrete locations, then returns over the winter to retrieve its cache. This improbable behavior requires the coordinated activity of different brain structures to integrate spatial coordinates encoded in the hippocampus with memories of how to find the seed stash. As it turns out, food-storing birds have a significantly larger hippocampus—a brain region involved in spatial organization and memory—than nonhording species.

In the laboratory, rats learning maze tasks also rely on hippocampal spatial information, which the prefrontal cortex integrates with memory of the route, task rules, and other relevant cues to direct navigational decisions. How the brain coordinates this activity is an active area of research. When neuron populations fire in sync, they produce oscillations in brain wave patterns (measured as local field potentials) that operate at many different frequencies. Brain wave frequencies called theta rhythms, which are prevalent in the rat hippocampus, are associated with working memory and decision-making in both animals and humans. Theta rhythms— which oscillate at about eight cycles per second—appear to act like a metronome for individual neurons that "phase lock" their firing in time with the theta rhythm.

DOI: 10.1371/journal.pbio.0030432.g001

As rats ran through this maze, their brain activity was recorded to study the neural basis of memory and decision-making.

Whether the synchronized activity of neuron populations across different brain structures correlates with functions like decision-making, and whether phase-locking somehow coordinates these diverse structures has remained an open question. But now, by training rats on a spatial working memory task—navigating a maze to a food reward—Matthew Jones and Matthew Wilson demonstrate a clear correlation between coordinated hippocampal and prefrontal cortex activity and memory or decision-making processes.

Jones and Wilson first trained rats on a simple maze task. The maze was shaped like a stretched-out "H" (see diagram). Rats were trained to shuttle back and forth across the long central arm for about 20 trials per day. At one end of this central arm, a moveable barrier directed the rats to turn either left or right toward a chocolate reward. At the opposite end, rats encountered a free choice at the T-junction: the correct turn (leading to more chocolate) was contingent upon the direction in which they were previously directed by the barrier at the "forced turn" end of the maze. As rats ran toward this choice point, they therefore had to "hold in mind" both task rules and information about the preceding forced turn in order to decide upon the correct route. Like the nutcracker, flying from seed stash to seed stash in search of its food, the rats' performance presumably relies upon coordination of spatial information stored in the hippocampus with connected brain regions that guide behavior.

After rats had learned to correctly navigate the maze over 80% of the time for two straight days, they were outfitted with electrodes to search for neurons showing task-related activity. The authors recorded action potentials (activation signals) of groups of individual neurons, and local field potentials, from the medial prefrontal cortex (mPFC), which is associated with working memory and decision-making, and a hippocampal region called CA1 (named after the Egyptian god Ammon's horns, *cornu Ammonis* in Latin). It has been known since the early seventies that neurons in CA1 show spatially selective activity—that is, each neuron fires action potentials only in restricted regions of an animal's environment.

Firing rates of individual neurons in both CA1 and mPFC were indeed task-related: they distinguished between the directions of runs across the central arm, and between the different routes between reward points during the choice stages. The firing rates of CA1–mPFC neuron pairs coactivated during central arm crossings showed the highest correlations as rats ran toward the decision point. This correlated activity between the neuron pairs was significantly reduced when rats made mistakes and chose the wrong direction. Such synchronized activity, the authors explain, may represent the transfer of spatial information from the hippocampus to a working memory system in the mPFC.

Jones and Wilson go on to show that many CA1 and mPFC neurons were phase-locked to theta rhythms, with enhanced phase-locking during trials requiring working memory and decision-making. This effectively means that the firing of neurons in both structures was aligned to the same theta rhythm "metronome." This, in turn, means that CA1–mPFC activities became correlated during distinct portions of the task. These correlations suggest that, as expected, the coordination and function of different brain regions depends on the task at hand. Additionally, this study shows that theta rhythms can be used as a reference against which to coordinate hippocampal and mPFC activity in accordance with behavioral demands of this maze task. Beyond shedding light on the neurobiology of behavior, these findings suggest that theta rhythms may contribute to diseases that involve disruptions in prefrontal cortex connectivity, such as schizophrenia—which, interestingly, can impair the spatial working memory of patients. —*Liza Gross*