

Synopses of Research Articles

A New Gene That Shapes Mouse Pigmentation Patterning

Scientists have long known that variation in animal color patterns carry far more than cosmetic significance. Darwin first connected pigmentation with adaptive advantage, noting that male finches with bright red plumage enjoyed greater reproductive success than their drab competitors. Explaining why coloration confers such advantages, however, has proved somewhat easier than showing how it arises. Biologists studying how neighboring regions of the vertebrate body plan develop differences in appearance and form have identified a small number of signaling pathways common to all animals. How and whether these pathways also control the developmental expression and variation of surface attributes like hair color, hair density, and hair length are unclear. By studying an old mouse mutant called *droopy ear*, Gregory Barsh and colleagues show that a member of the well-known family of T-box genes is required for a key pigmentation pattern in mice.

Many vertebrate species—be they fish, bird, or mammal—have a much lighter belly than back. Studies in mice indicate these dorsoventral pigment differences arise from differential expression of the *Agouti* gene in the ventral and dorsal regions of the developing mouse; *Agouti* produces a pale yellow color and thus mice with light bellies have *Agouti* expressed in their ventral but not dorsal region. *Droopy ear* was discovered more than 50 years ago by virtue of its effects on head and ear shape, but it also affects pigmentation patterns; mutant mice have expanded ventral-specific *Agouti* expression into the dorsal region.

First, Sophie Candille, a graduate student in Barsh's laboratory, searched for the gene that underlies the defect in *droopy ear*. When the researchers homed in on the chromosomal region known to harbor *droopy ear*, they found *Tbx15*—a member of the T-box gene family. T-box genes are found in a wide range of species and play diverse roles during embryonic development. In the *droopy ear* mouse, *Tbx15* carries a mutation that makes

the protein nonfunctional. The researchers made certain that *Tbx15* really is the *droopy ear* gene by deleting most of the gene's coding region and showing that this "knocked-out" gene produces the typical *droopy ear* mouse.

The pattern of embryonic *Tbx15* expression—determined by observing messenger RNA transcripts in developing tissues of the head, trunk, and limbs—suggests that early expression of *Tbx15* in the dorsal flank sets coordinates for dorsoventral differences in hair length and pigmentation. Candille et al. demonstrate that the regional pigment differences characteristic of adults is indeed established soon after embryonic *Tbx15* expression. So this boundary in pigmentation is set up very early during development. Interestingly, the early coordinates of the future pigment boundary do not correspond to any other known developmental boundary.

The *Tbx15* pigmentation effects seen in these lab mice, the researchers note, resembles coat variations in other mammals, including German shepherds and an endangered mouse whose

lighter dorsal markings once gave it an adaptive advantage on the white sand reefs where it lives (sadly, such markings offer no protection against loss of habitat). T-box genes are also found in humans; mutations in *Tbx1*, *Tbx4*, *Tbx5*, and *Tbx22* can cause developmental abnormalities of the heart, limbs, or of the head and neck. Mutations of human *Tbx15* have not yet been identified, but could contribute to regional differences of pigmentation (in dorsal and ventral surfaces of the limbs, for example) or to development of the head and neck. The identification of *Tbx15* adds a new player to the genes that help pattern the developing embryo—attention now turns to the controls that regulate *Tbx15* and the *Tbx15* targets, which set up the pattern.

Candille SI, Van Raamsdonk CD, Chen C, Kuijper S, Chen-Tsai Y, et al. (2004) Dorsoventral patterning of the mouse coat by *Tbx15*. DOI: 10.1371/journal.pbio.00020003



Dorsoventral pigment boundaries in mouse and human

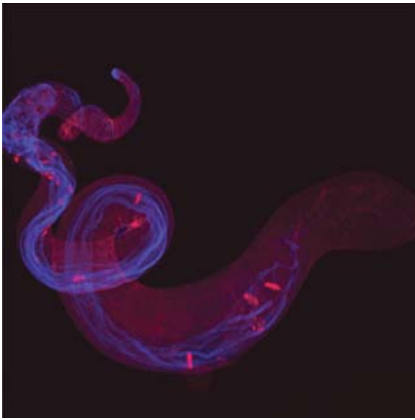
"Suicide" Proteins Contribute to Sperm Creation

You might say that caspases are obsessed with death. The primary agents of programmed cell death, or apoptosis, caspases kill cells by destroying proteins that sustain cellular processes. Apoptosis, a highly controlled sequence of events that eliminates dangerous or unnecessary cells, contributes to a wide variety of developmental and physiological processes—in a developing embryo, apoptosis creates the space

between fingers and adjusts nerve cell populations to match the number of cells they target; in an adult, apoptosis counters cell proliferation to maintain tissue size and density. Now it appears that caspases may also play a role in creating life. As Bruce Hay, Jun Huh, and colleagues report, multiple caspases and caspase regulators are required for the proper formation of free-swimming sperm in the fruitfly *Drosophila*.

Caspases, which typically exist in a quiescent state in nearly all cells, are regulated through a complex network of activators and inhibitors. Once activated, a "caspase cascade" ultimately cleaves and irreversibly alters the function of essential cellular proteins, leading to apoptosis. A few of the dozen-plus known caspases appear to contribute to inflammation responses, but the vast majority are enlisted to





Developing spermatids in a normal *Drosophila* testis

kill cells. Not surprisingly, cells keep caspase activation under tight wraps. That's why it's intriguing that multiple caspases normally associated with the induction of cell death participate in this nonapoptotic process.

During spermatogenesis, germline precursor cells—the cells that generate sex cells—give rise to 64 haploid spermatids. (Sex cells are haploid, containing half the chromosomes found in body cells.) Spermatids are connected by intracellular “bridges” that, along with most other cytoplasmic components, must be expelled in a process called “individualization” to create terminally differentiated free-swimming sperm. Protein structures known as investment cones surround each spermatid nucleus and sweep out the neighboring cytoplasm, bridges, and organelles, forming a bulge that eventually detaches as a “waste bag” as it reaches the sperm

tail. This process—elimination of cytoplasm and membrane packaging of individual spermatids—also occurs in mammals. Many types of human infertility result when it is disrupted.

To explore how caspases affect this process, Hay's group studied the consequences of inhibiting caspase activity (or the activity of specific caspase activators) in the male germline cells of fruitflies. In both cases, they observed that the bulges and waste bags were either abnormally small or absent and that the normal path of investment cone movement was disrupted. The researchers also inspected the flies to look for structural differences and found that spermatids in both mutant strains remained connected by cytoplasmic bridges and retained residual cytoplasm. Together, the authors conclude, these results demonstrate that individualization depends on caspase activity.

Hay's team went on to characterize the pathways that activate caspases during sperm individualization. They found that in one pathway, two key activators of caspase-dependent cell death—Ark and Hid (both of which have mammalian counterparts)—promote the activity or stabilization of the caspase Dronc. A second caspase, Dredd, and its activator Fadd (which also have mammalian counterparts) were also found to be important. Double mutants that removed both Dronc and Dredd activity had more severe defects in individualization than mutants that removed only one or the other, suggesting that these caspases have distinct roles in this process. Interestingly, Drice—the downstream

caspase activated just as individualization begins (downstream caspases are typically activated by upstream caspases such as Dronc and Dredd)—was not affected by inhibition of Dronc and Dredd. This result, along with the fact that Dronc and Drice were activated at different times and places, suggests that some other mechanism activates Drice. Different apoptosis-related caspases and caspase regulators, the authors conclude, are recruited through different pathways at distinct points in time and space to create individually packaged, free-swimming sperm, a distinctly nonapoptotic process.

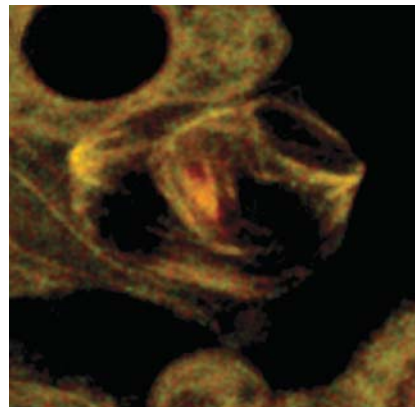
Studies in mice suggest that individualization may occur similarly in mammals, with activation of apoptotic caspase cascades resulting in free-swimming sperm and loss of specific caspase activators causing infertility and defective spermatogenesis. The abnormal differentiation and residual cytoplasm seen in caspase-inhibited *Drosophila* mutants, for example, resemble “cytoplasmic droplet sperm,” a condition seen in infertile men. Insights into the molecular basis of caspase activation in sperm individuation could provide clues to male infertility and suggest possible treatments. Given the widespread role of programmed cell death in supporting processes fundamental to life, perhaps it's not surprising that the agents of apoptosis also support the creation of life.

Huh JR, Vernooij SY, Yu H, Yan N, Shi Y, et al. (2003) Multiple apoptotic caspase cascades are required in nonapoptotic roles for *Drosophila* spermatid individualization. DOI: 10.1371/journal.pbio.0020015

Visualizing Noncentrosomal Microtubules during Spindle Assembly

As cells can only arise from cells that already exist, continuity of life depends on the highly regulated sequence of events that control cell division. This process is mediated by a complex macromolecular structure called the mitotic spindle. The most conspicuous components of the spindle are microtubules, which are made of tubulin and other associated proteins. In most animal cells—body cells and male germline cells (spermatocytes)—spindle assembly is orchestrated by organelles called centrosomes, which actively polymerize (that is, add tubulin subunits) and stabilize microtubules. The spindles found in these cells are known as *astral* because of the star-shaped asters—structures made of centrosome-anchored microtubules—that can be observed

associating with each spindle pole. Some cells—such as the cells of the female germline (oocytes)—do not contain centrosomes, and the chromosomes themselves seem to arrange and stabilize the microtubules into spindles. These spindles are referred to as *anastral*.



Centrosome-independent spindle assembly

To gain insight into the mechanisms of spindle assembly, scientists are increasingly relying on techniques that allow them to directly observe dynamic, complex processes in the living cell. Using time-lapse microscopy of fluorescently labeled fruitfly (*Drosophila melanogaster*) spermatocytes, Cayetano Gonzalez and his colleagues at the European Molecular Biology Laboratory in Germany (and now at the Centro Nacional de Investigaciones Oncológicas in Spain) have been able to observe the



assembly and sorting of microtubules of noncentrosomal origin in cells that contain centrosomes. The task of flagging such microtubules is complicated by the fact that centrosomes become quite active microtubule organizers once cell division begins. Thus, as soon as the membrane around the nucleus breaks down, microtubules from the centrosome invade the nuclear region, making it hard to identify any noncentrosomal microtubules that might appear. To get around this problem, Elena Rebollo in the Gonzalez lab set up two experimental conditions under which centrosomes remain functional but are kept affixed to the cell membrane—and, therefore, away from the nucleus—in *Drosophila* spermatocytes. One takes advantage of a genetic mutation (called *asp*, for abnormal spindle); the other uses a transient treatment with a drug (called colcemid) that depolymerizes microtubules.

In these modified cells, microtubules can be seen growing not only over the membrane-bound centrosomes, as expected, but also over the nuclear region, away from the centrosomes. Nucleation, or formation, of such noncentrosomal microtubules has a relatively late onset, starting only once chromosomes are condensed, and takes place on the inner side of the remnants of the nuclear envelope. In a fraction of cells, these microtubules are sorted into bipolar spindle-shaped structures, highly reminiscent

of the anastral spindles found in oocytes. Chromosome segregation—a critical stage of cell division—and cell division itself tend to be aberrant in these cells.

These results, Rebollo et al. propose, strongly suggest that microtubules of noncentrosomal origin may significantly contribute to spindle assembly even in cells that contain active centrosomes. Moreover, by facilitating the nucleation of such noncentrosomal microtubules, the degraded nuclear envelope may play a previously unsuspected role in spindle assembly in *Drosophila* spermatocytes. It is unlikely, the researchers also conclude, that the anastral spindles they have observed can fill in as a backup to ensure successful cell division. More likely, they argue, both centrosomal and noncentrosomal microtubules are required for proper spindle assembly and robust cell division in cells with centrosomes. As the authors point out, *Drosophila* is a rich model system that should help scientists further investigate the intricacies of spindle assembly. The answers will help us understand how the cell executes one of its most important duties: safeguarding genomic stability for future generations.

Rebollo E, Llamazares S, Reina J, Gonzalez C (2004) Contribution of noncentrosomal microtubules to spindle assembly in *Drosophila* spermatocytes. DOI: 10.1371/journal.pbio.0020008

Mechanism Suggests How HIV Protein Disrupts Immune Cell Migration

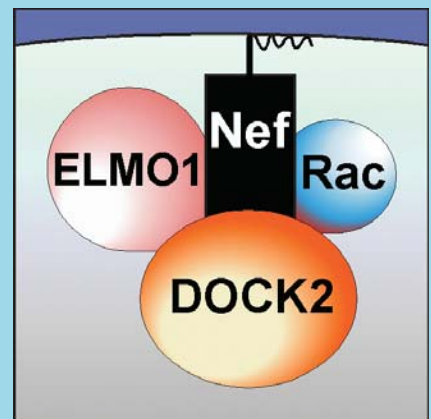
One of the cornerstones of immune system function is movement. When word spreads that a virus has entered the body, chemical signals tell lymphocytes to proliferate and travel to the site of infection. Efforts to combat HIV have focused on understanding how the virus disrupts this immune response in the hopes of developing drugs to block its replication as well as vaccines to control the virus itself. Toward this end, scientists are investigating how each of the virus's nine genes—which all appear to have multiple functions—contribute to HIV infection.

When HIV infects a cell, viral enzymes copy its RNA genes into DNA, which can then invade the infected cell's chromosomes. The viral DNA might lay dormant or it might use the cell to reproduce more viruses, which go on to infect other cells. The course of infection is determined by interactions between circulating T cells and antigen-presenting cells (cells that present evidence of infection), like macrophages, which may unwittingly aid the virus by transferring it to the T cells. Macrophages, for example, produce proteins that tell T cells to come check out an infection.

A viral protein called Nef sparked intensive research after observations that patients with a rare strain of HIV lacking Nef took a very long time to develop AIDS symptoms. Nef has been linked to molecules involved in macrophage- and other antigen-signaling pathways and may use the molecules to appropriate these pathways for its own ends—enhancing virulence by facilitating viral replication. How Nef does this is not entirely clear. Now Jacek Skowronski and his colleagues at Cold Spring Harbor Laboratory in New York have identified the key molecules that Nef enlists to co-opt the signaling machinery of immune cells.

To understand how this might happen, biochemically speaking, Skowronski's lab first needed to determine which molecules Nef associates with. An adaptor protein, Nef does not directly catalyze reactions, but binds to enzymes that do. The researchers identified two proteins, DOCK2 and ELMO1, that form a complex with Nef. DOCK2 regulates enzymes, called Rac1 and Rac2, that are required for normal lymphocyte migration and antigen-specific responses. ELMO1 has also been shown

to help DOCK2 activate Rac. Because DOCK2 activates Rac as part of two different signaling pathways—one activated by the T cell receptor, which mediates T cell activation, and one by a chemokine receptor, which controls T cell



Pathway by which Nef disrupts T cell migration

migration—the researchers investigated whether Nef could affect these important pathways by modulating Rac activity. They found that Nef in fact activates Rac by binding to the DOCK2–ELMO1 complex. And they went on to

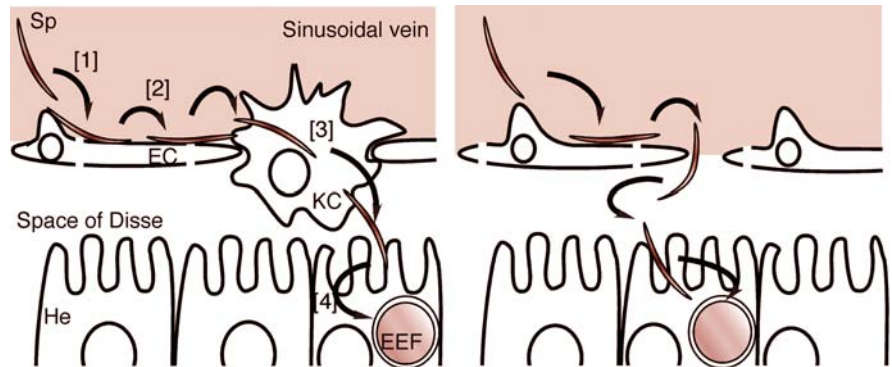
Protein Essential for Malarial Parasite to Reach and Infect Liver Cells

Plasmodium, the microscopic parasite that causes malaria, passes through two hosts, two reproductive modes, four habitats, and over half-a-dozen distinct developmental stages in one lifecycle. When a *Plasmodium*-infected mosquito bites a human, it injects the parasite—sequestered in the mosquito's salivary glands in its sporozoite stage—into the victim's bloodstream. Within hours, the sporozoites invade the liver—a critical stage for establishing infection—and spend the next few weeks asexually dividing inside liver cells, eventually releasing thousands of merozoites into the bloodstream. Merozoites quickly invade red blood cells and begin a second round of asexual proliferation. The infected cells rupture and die, releasing more parasites and toxins. The toxins cause malaria's characteristic fever and chills, and the liberated merozoites initiate another cycle of red blood cell attacks.

show that HIV uses these components of the chemokine receptor pathway to disrupt T cell migration. To generate an effective immune response, it is crucial that T cells travel to sites within lymphatic tissues where they interact with other lymphocytes. By inhibiting T cell migration, the researchers propose, Nef prevents these critical interactions, thereby providing a mechanism for stifling the immune response.

These results, the authors argue, provide the biochemical evidence that Nef targets a protein “switch” that can interfere with important aspects of T cell function. In this way, Nef subverts the immune response pathways controlled by receptors on the surface of T cells to effectively disarm the immune system and turn T cells into viral replication factories. Understanding how Nef interacts with these proteins to spread infection could lay the foundation for valuable new therapies aimed at inhibiting and arresting HIV infection by blocking Nef-mediated effects.

Janardhan A, Swigut T, Hill B, Myers MP, Skowronski J (2004) HIV-1 Nef binds the DOCK2–ELMO1 complex to activate Rac and inhibit lymphocyte chemotaxis. DOI: 10.1371/journal.pbio.0020006



Sporozoite migration to hepatocytes

An unresolved question has been how the circulating sporozoites reach the liver cells in the first place, since liver cells are separated from the bloodstream by a layer of endothelial and Kupffer cells, which form the walls of the liver capillaries. (Kupffer cells project into the bloodstream and remove contaminants.) Having identified a protein required for sporozoite migration through the capillary lining, Tomoko Ishino, Masao Yuda, and their colleagues at Mie University School of Medicine in Japan may have found an answer.

Only four of the roughly 150 vertebrate-infecting *Plasmodium* species affect humans. *P. falciparum*, the most pathogenic of the human-infecting species, is closely related to avian and rodent species. One rodent species, —*P. berghei*—shares fundamental aspects of structure, physiology, and lifecycle with *P. falciparum* and so serves as a model for the human parasites. Since sporozoites must infect mosquito salivary glands before they can infect the mammalian liver, Yuda's team searched for sporozoite genes that are predicted to encode secretory or membrane proteins and are expressed only in mosquito salivary glands. Their search revealed a coding region conserved in several species of *Plasmodium*.

Tracing the gene's activity through the parasite's life cycle, Yuda's team confirmed that it was expressed only in sporozoites in the mosquito salivary gland—not in the mosquito midgut, where sporozoites are produced after mosquitoes feed on the blood of an infected person. The corresponding protein was localized to micronemes, specialized secretory organelles found at the front end of malaria parasites. Because micronemes

are known to play a central role in *Plasmodium* motility and invasion, the researchers predicted this protein would also be important in migrating to or invading liver cells. They named the protein SPECT, for sporozoite microneme protein essential for cell traversal.

Yuda's team tested SPECT's function by generating *spect*-disrupted mutants and observing how the altered parasites affected their hosts. *spect* disruption did not affect parasite proliferation in rat red blood cells or interfere with parasite development in the mosquito midgut or salivary glands, but it did have an effect on the parasite's ability to infect the liver. Rats injected with *spect*-disrupted parasites had significantly lower levels of liver infection than rats injected with nonmutant parasites. Since it was unclear whether the *spect*-disrupted mutants lost their infectivity or simply could not pass through the cell layer, the researchers inoculated human liver cells with the mutants and found that they infected the cells normally.

Yuda's team also tested SPECT's impact on sporozoite cell-passage ability; if the mutants couldn't reach the liver cells, they couldn't infect them. *spect*-disrupted parasites completely lost their ability to pass through cells. Since traversal of the cellular barrier between liver cells and the circulatory system is a crucial step in malarial infection, the authors conclude, SPECT and other proteins involved in shuttling sporozoites into liver cells could be effective targets for malaria treatment and prevention.

Ishino T, Yano K, Chinzei Y, Yuda M (2004) Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer. DOI: 10.1371/journal.pbio.0020004



A Truly Broad View of Gene Expression Spotlights Evolution and Diversity

Bioinformatics and microarrays have given scientists powerful new tools to investigate the structure and activity of genes on a global scale. Rather than studying just a few genes, scientists can analyze tens of thousands within and across species. Microarrays flag which genes are expressed under particular cellular conditions in an organism, while genome sequencing offers clues to gene function and regulation.

By comparing the genomic properties of different species, scientists can spot patterns that help them identify functional and regulatory elements, learn about genome structure and organization, and gain a better understanding of the evolutionary forces that shape life on Earth.

The potential of these technologies to reveal insights into the fundamental structure and function of biological systems continues to grow along with the wealth of gene sequence and expression data—but the ability to interpret and merge these datasets lags behind the ability to collect them.

In an effort to overcome these limitations, Sven Bergmann, Jan Ihmels, and Naama Barkai developed a comparative model that integrates gene expression data with genomic sequence information.

Because functionally related genes are expected to be coexpressed in different organisms and because the sequence of some of these functionally related genes may also be conserved between organisms, Bergmann and colleagues hypothesized that “conserved coexpression” could serve as an indicator of gene function on a genomic level. (Conserved genes are those that have changed little since they first evolved. Conserved coexpression describes functionally related genes that are activated together in different species.) But first they had to determine whether coexpression was conserved among species. Analyzing the gene expression profiles of six distantly related organisms—bacteria, yeast, plant, worm, fruitfly, and human—the researchers found that functionally related genes were indeed coexpressed in each species. The most strongly conserved sets of coexpressed genes are associated with core cellular processes or organelles. These results indicate that conserved coexpression can improve the interpretation of genome sequence data by providing another functional indicator for homologous sequences.

Since functionally related genes are expressed together in different organisms, it would be reasonable to think their regulatory networks are also conserved. To explore this idea, the researchers grouped coexpressed genes and their regulatory elements into “transcription modules” for each organism. They found significant variation in the number, organization, and relative importance of these modular components. Which components contributed most to an organism’s global transcription program, for example, depended on the organism. But they also found that the transcription networks are highly clustered—meaning that genes connected to a specific gene are also connected to each other. This finding indicates that gene expression programs, regardless of their size or individual

components, are highly modular. Each transcriptome contains modules that have been conserved over time along with “add-on” modules that reflect the needs of a particular species. This modularity supports the notion that variation between and among species arises from the diversity of gene expression programs.

Although the regulatory details of individual gene groups varied, the researchers found common ground in the overall landscape of the expression data. The transcription programs exhibit properties typical of dynamically evolving “real-world” networks that are designed to perform in uncertain environments and to maintain connections between elements independent of scale. These properties were originally identified in studies of social networks and the World Wide Web, but they aptly describe the real-world challenges of the cell. Studies of dynamically evolving networks show that nodes

(i.e., genes and proteins) added at an early stage (much like highly conserved genes) are more likely to develop many connections, acting as a hub. Following these organizational principles, transcription networks would have a relatively small number of highly connected “hub genes”—though a much higher number than one would expect in a random network.

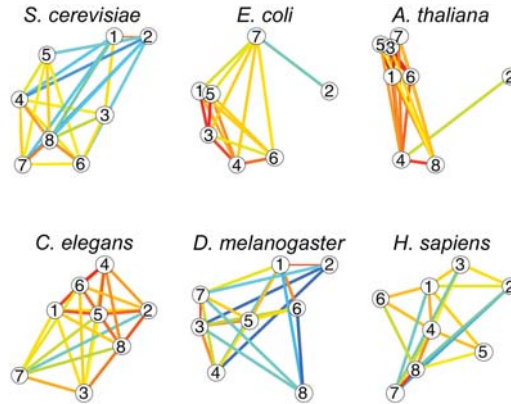
And that is what the authors observed: the networks they constructed from the expression data had the expected number of highly connected hub genes, which tend to be essential and conserved among organisms. Since these highly connected genes are likely to have homologues in other organisms, they can serve as powerful and efficient tools for assigning function to the thousands of uncharacterized sequences found in sequence databases. This model presents a framework to explore the underlying properties that govern the design and function of the cell and provides important clues—in the form of conserved transcription modules—to the evolutionary building blocks that generate diversity.

Bergmann S, Ihmels J, Barkai N (2003) Similarities and differences in genome-wide expression data of six organisms. DOI: 10.1371/journal.pbio.0020009

Researchers Add to Proteomics Toolbox

Genes use a simple language—written in the molecules of DNA—to build thousands of proteins in a dizzying variety of sizes and shapes. With only four different nucleotide building blocks, DNA codes for the 20 different amino acids (each with their own structures and properties) that provide the foundation for the enormous diversity of protein form and function. This diversity makes the systematic study of all the proteins of a given organism (called proteomics), a challenging enterprise.

Interactions between proteins underlie nearly every fundamental process within the cell. They can form higher-order multiprotein complexes like those involved in transcription and replication, help transport proteins to their proper location in



Regulatory relations among transcription modules

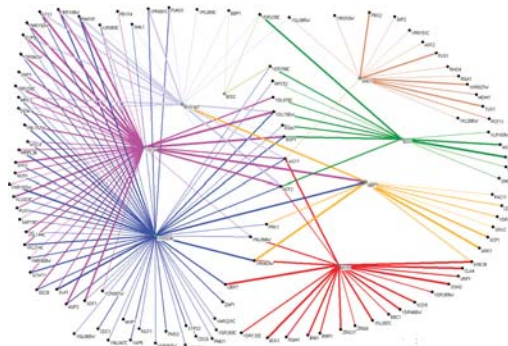
the cell, and participate in signaling pathways. Because of their importance, disruption of these interactions can have disastrous consequences. For example, the loss of the ability of a normal cellular protein called Src to bind to certain other proteins can be associated with cancer progression. The determinants of these interactions are poorly understood, but in many cases these interactions are mediated by small pieces of the proteins, which are called peptides. Peptides serve as the starting point for the novel strategy reported in this issue.

Gianni Cesareni and colleagues have added to the repertoire of proteomic analysis by devising a global strategy to investigate protein–protein interactions on an organismal level using yeast as a model organism. The authors select a protein of interest from yeast, which can be thought of as the “bait” for which they wish to identify protein-binding partners. They start by looking at a number of different previously identified peptides that bind the bait protein. Commonalities between the sequences of these peptides form the “consensus” binding sequence, a base framework of protein sequence from which many possible variations can be derived. Since the protein sequences of all proteins (the proteome) in yeast can be deduced from the sequenced genome, the authors can scan the proteome for proteins that contain the consensus, or a closely related, sequence. These proteins could potentially bind the bait

protein. Peptide sequences from these identified proteins are synthesized chemically and arrayed on a membrane, which is bathed in a solution containing the bait protein. After washing off the excess bait protein, they can figure out where it remains on the membrane and therefore tell which peptides the bait protein has bound. The proteins corresponding to these peptides are candidate binding proteins that are validated by further experimentation.

The protein–protein interactions identified by this approach can be used to extend the network of known interactions in the proteome. This will enable researchers to draw functional linkages between proteins, whether they are involved in a basic biological process or in human disease. By examining whole families of proteins, it may also aid in elucidating the underlying determinants of binding specificity, which would provide clues to the biomechanisms underlying cell processes. These insights could lead to methods for manipulating these interactions, perhaps even in cases of human disease, as in the case of Src and cancer. This approach can readily be applied to the proteomes of more complex organisms like humans and adds to the growing number of experimental strategies available to researchers in proteomics.

Landgraf C, Panni S, Montecchi-Palazzi L, Castagnoli L, Schneider-Mergener J, et al. (2004) Protein interaction networks by proteome peptide scanning. DOI: 10.1371/journal.pbio.0020014



Protein interaction network

A DNA-Binding Protein Helps Repair Breaks in DNA Double Helix

One of the central problems for much of the 20th century was how to reconcile genetic stability with evolutionary change. Genomic fidelity was thought to arise from an inherent invariability in the DNA structure itself. Biologists now know that DNA constantly undergoes modifications as it unwinds, replicates, condenses, twists, and untwists. This dynamic interplay produces both stability and variation—and occasionally genetic damage. If DNA damage goes unrepaired, it can disrupt chromosomal integrity and may lead to cancer and other diseases. When the DNA double helix breaks, the cell must enlist a number of proteins to repair the broken DNA ends, but much remains to be learned about the molecular mechanisms involved. Tracking a protein that binds to single strands of DNA during replication and recombination in living yeast cells, Xuan Wang and James Haber report that this protein plays a role in at least two key steps in the repair of double-strand breaks in DNA.

When double-strand breaks occur,

the cell mounts a search for similar (homologous) sequences that can be used as a template to repair the damaged sequence. If successful, the broken DNA molecule basepairs with the homologous region and forms a complex, ultimately replacing the damaged sequence with a similar sequence. In yeast—which serves as a stand-in for higher eukaryotes, including humans—this “strand invasion” process requires both an exchange protein, called Rad51, and a single-stranded DNA-binding protein, called RPA (replication protein A). Single-stranded binding proteins bind to regions of DNA that are opened up during replication. They also bind to strands when broken ends of DNA are cut by enzymes that leave long single-stranded tails. RPA proteins are thought to facilitate the formation of Rad51 polymers, or filaments, on single-stranded DNA by clearing away structures that block Rad51’s path. The growing filament searches for homologous DNA sequences and promotes the invasion

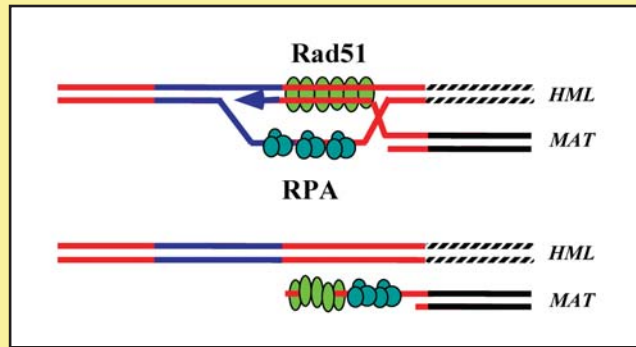
of the single strand, preparing it to copy the homologous template by “repair DNA synthesis,” which patches up the lesion.

To investigate how RPA functions in double-strand break repair in a living organism, Wang and Haber created cells with a double-strand break at a specific site and monitored the activity of proteins recruited to repair the damage. With this approach, the researchers could observe these interactions in living yeast to determine what role RPA plays in repairing DNA damage and how it works with the Rad51 protein.

The authors show that as soon as a double-strand break occurs, the RPA protein binds to the exposed strand ends, before the Rad51 protein does. This is not unexpected, because this binding order supports the model that RPA prepares the way for Rad51, perhaps by stabilizing the strand long enough for Rad51 filaments to establish themselves. The surprise was that RPA appears to be necessary even after Rad51 binds to the DNA strand, perhaps by stabilizing the

interaction with homologous DNA sequences. That RPA is required for successful repair is supported by evidence that a particular mutated form of RPA can stimulate Rad51 DNA binding normally, but inhibits strand exchange and template copying, thus preventing repair of DNA damage.

Wang and Haber's work highlights the complex repertoire of DNA-protein and protein-protein interactions



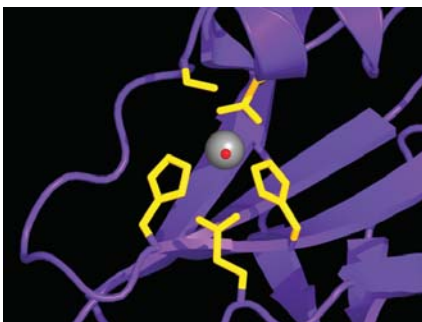
Repair of double-strand breaks involves invasion of the homologous region, displacement, and DNA synthesis to fill in the gap

that manage and manipulate the genome in the service of genomic stability. The study of DNA repair mechanisms in living cells—a daunting task—promises to lend valuable insights into the truly dynamic nature of maintaining genome stability.

Wang X, Haber JE (2004) Role of *Saccharomyces* single-stranded DNA-binding protein RPA in the strand invasion step of double-strand break repair. DOI: 10.1371/journal.pbio.0020021

Structure and Implications of JAMM, a Novel Metalloprotease

Proteins may be the workhorse of the cell, but when a cell can synthesize one protein in a matter of minutes, chances are some will become obsolete. Though many proteins put in years of productive service, others quickly outlive their usefulness and can even damage the cell. Proteins that help form bone and muscle, for example, function for years while regulators of mitosis and cell proliferation might finish their jobs in seconds. Such short-timers are soon tagged as superfluous by a chain of small proteins called ubiquitin, which marks the proteins for degradation in an enzyme called the proteasome. Once in the proteasome, these proteins are broken down and can then be recycled for more productive ventures.



The active site of JAMM

A massive structure by cellular standards, the proteasome consists of multiple subunits, including a cylindrical core particle called 20S, which catalyzes degradation, and regulatory complexes called 19S caps, which form lid and base structures at both ends of the core. While the structure and biomechanics of the 20S core have been well characterized, much less is known about the functional mechanics of the regulatory complexes. The lid-base complex recognizes only ubiquitin-tagged proteins, which are then unfolded so they can enter the proteasome. But first ubiquitin chains must be detached from the protein, a task performed by an enzyme in the proteasome called Rpn11 isopeptidase. How the lid-base complex removes the ubiquitin tag, unfolds the protein, and shuttles it into the proteasome's core is not clear. Now Raymond Deshaies and colleagues present the structure of a homolog of the 19S lid's isopeptidase enzymatic center and provide new insights into these questions.

The proteasome Rpn11 subunit contains a key region called the JAMM motif, which Deshaies' lab has shown previously is required for the proteasome to remove ubiquitin tags. For the work discussed in this paper, the researchers set out to understand how the proteasome strips off ubiquitin tags from proteins about to be destroyed by determining the three-dimensional structure of the JAMM motif.

The researchers tested many genes to look for a JAMM-containing protein that would crystallize properly and found one in the heat-loving prokaryote *Archaeoglobus fulgidus*. After determining the structure of the JAMM protein (called AfJAMM), the researchers discovered that AfJAMM looks nothing like the well-known deubiquitinating enzymes. But the arrangement of a set of amino acids that binds a zinc ion and forms the proposed active site of AfJAMM does resemble that found in a well-known protein-degrading metalloprotease called thermolysin, even though in other respects AfJAMM and thermolysin have very different features. The researchers mutated amino acid residues in another JAMM protein called Csn5 (they expected these residues to be critical for isopeptidase activity as well, based on comparisons of the AfJAMM and thermolysin structures) and found that the residues are indeed important for Csn5 function. These results suggest that JAMM does indeed represent a novel family of metalloproteases.

As for the wider function of JAMM proteins, the researchers speculate that these proteins are likely to be involved in a variety of important regulatory systems since they appear in life forms that lack ubiquitin and ubiquitin-like proteins. The crystal structure reported in this paper will provide a valuable tool for investigations into the underlying structural and functional mechanisms of these enzymes. And it may have important therapeutic implications. Proteasome inhibitors are promising anticancer therapies—fighting cancer by blocking machinery required by rapidly dividing cells. In the hopes of developing more targeted therapies, scientists are trying to fine-tune their control of the ubiquitin system and the proteasome. Inhibiting the JAMM domain of enzymes like Csn5, which remove ubiquitin-like tags from proteins upstream of the proteasome, for example, might just do the trick.

Ambroggio XI, Rees DC, Deshaies RJ (2003) JAMM: A metalloprotease-like zinc site in the proteasome and signalosome. DOI: 10.1371/journal.pbio.0020002

Evolution of Primate Sense of Smell and Full Trichromatic Color Vision

Conventional wisdom says that people deficient in one sense—such as vision or hearing—often acquire heightened acuity in another. And some studies support this notion by showing that areas of the brain known to control vision can respond to other forms of sensory stimuli in persons



Howler monkey

without sight. These adjustments, of course, take place over the lifetime of an individual. Now it appears that similar adjustments may occur over evolutionary time. Investigating the deterioration of olfactory receptor (OR) genes in primates, Yoav Gilad and his colleagues at the Max Planck Institute for Evolutionary Anthropology in Germany and the Weizmann Institute in Israel found a correlation between the loss of OR genes and the acquisition of full trichromatic color vision.

OR genes—the molecular basis for the sense of smell—form the largest gene superfamily in mammalian genomes. But a high percentage of these genes are “pseudogenes,” DNA sequences that are remnants of genes that are no longer functional. Following an evolutionary “use-it-or-lose-it” rule, pseudogenes tend to evolve in larger gene families where there’s no selective advantage in having, say, 100 versus 120 genes. While humans, nonhuman primates, and mice have

roughly the same number of OR genes, in humans a much higher percentage of these are pseudogenes, at 60%, while nonhuman apes have about 30%, and the mouse has about 20%. Reliance on the sense of smell, it appears, decreases for animals that develop a dependence on other senses, such as hearing or sight, to survive. In characterizing this high proportion of pseudogenes, Yoav Gilad et al. asked: Is this characteristic of all primates? If not, at what point in primate evolution did the increase occur?

Looking at 19 primate species—including one human, four apes, six Old World monkeys, seven New World monkeys, and one prosimian—Gilad et al. randomly sequenced 100 distinct OR genes from each of the species. The team found that Old World monkeys had roughly the same percentage of OR pseudogenes as nonhuman apes, but a much higher percentage than New World monkeys—except for one, the howler monkey. The percentage of OR pseudogenes in the howler monkey was much closer to that seen in the Old World monkeys and apes than in its New World cousins. The sense of smell, it appears, deteriorated both in the ape and Old World monkey lineage and in the howler monkey lineage. Since Old World monkeys, apes, and the howler monkey do not share an exclusive common ancestor, this deterioration must have evolved independently in both groups. Surprisingly, howler monkeys share another sensory feature with apes and Old World monkeys: trichromatic color vision.

In trichromatic color vision, three retinal protein pigments, called opsins, absorb various wavelengths of light, which the brain processes to produce full-color images. Apes and Old World monkeys carry three opsin genes, and most New World monkeys carry only two, though females can sometimes have three. Only howler monkeys routinely have three genes occurring in both sexes. Thus, full trichromatic vision evolved twice in primates—once in the common

ancestor of apes and Old World monkeys, about 23 million years ago, and once in the howler monkey lineage, about 7–16 million years ago. The evolution of color vision, the authors propose, coincided with a growing complement of OR pseudogenes and a deterioration of the sense of smell. Gilad et al. suggest that investigating the types of visual cues required for finding food may shed light on the nature of this connection.

Gilad Y, Wiebe V, Przeworski M, Lancet D, Pääbo S (2004) Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. DOI: 10.1371/journal.pbio.0020005

Brain Activity during Slow-Wave Sleep Points to Mechanism for Memory

How does your brain pass the time while you’re sleeping? If you’ve ever wrestled the demons of insomnia, you know what sleepless nights can do to your mental agility. Sleep cycles in mammals are characterized by two distinct, successive sleep stages: slow wave and rapid eye movement (REM). Both stages of sleep have uniquely associated electrical activity in the brain, which neuroscientists can measure by placing electrodes on the brain during sleeping and waking states. What neuroscientists can’t easily measure is the purpose of these two sequential sleep stages.

The notion that sleep helps to improve memory was introduced over 80 years ago. Since then, several studies have demonstrated that sleep deprivation following the acquisition of a new memory strongly impairs its consolidation. Insight into the mechanisms underlying this effect came from the observation that neuronal activity patterns detected during waking reappear during ensuing sleep, suggesting that newly acquired “memory traces” may be replayed in the brain to solidify neural connections and thus “consolidate” memory. These reverberating patterns of activity have been observed in both mammals and birds, pointing to a very general biological phenomenon.

Still, the relationship between brain reverberation and memory consolidation

remains unclear for a number of reasons. First, studies to date have observed only subtle, short-lived reverberations lasting less than an hour and can't explain the memory-disrupting effects of sleep deprivation applied several hours and even days after initial memory encoding. And since brain reverberation in mammals has only been investigated in the hippocampus and cerebral cortex, it is unclear whether the phenomenon is specific to this neural circuit or is a more general property of the brain. Furthermore, reverberation studies have so far relied on neural activity measured in animals that were highly trained on specific laboratory tasks and therefore may simply not be representative of the acquisition of new memories. Finally, experience-dependent neural reverberation has been detected in both phases of sleep as well as waking, but no quantitative comparison of the different contributions of each state has been established.

In a study designed to address these concerns, Sidarta Ribeiro and his colleagues at Duke University in Durham, North Carolina, recorded over a hundred neurons continuously over the course of the normal sleep-wake cycle in rats, focusing on four major forebrain areas that are essential for rodent-specific behaviors. Halfway through the recording time, animals were transiently allowed to explore four strictly novel objects, each of them designed to provide different spatial and sensory cues. The



Sleeping rats

researchers found that in all the forebrain areas examined the neuronal firing patterns recorded when the rats initially explored the new objects reverberated for up to 48 hours after these objects were removed. The reverberation of neuronal activity sampled when rats explored familiar environs was insignificant. Reverberation was most significant during slow-wave sleep (a state that accounts for nearly 40% of a rat's life), decreased during waking periods, and was highly variable during REM sleep.

In this study, Ribeiro et al. demonstrate that long-lasting neuronal reverberation following novel waking experiences can occur in several forebrain sites and is strongly enhanced during slow-wave sleep. Because neuronal reverberations are sustained for long periods, this may provide a mechanism to recall and amplify memories until they are effectively stored. On the basis of differences observed between REM and slow-wave sleep in this and previous studies, the authors propose that the two phases of sleep play separate and complementary roles in memory consolidation. Thus, the two stages of sleep give the brain a chance to process the novel events of the day in peace.

Ribeiro R, Gervasoni D, Soares ES, Zhou Y, Lin S-C, et al. (2004) Long-lasting novelty-induced neuronal reverberation during slow-wave sleep in multiple forebrain areas. DOI: 10.1371/journal.pbio.0020024