Synopses of Research Articles

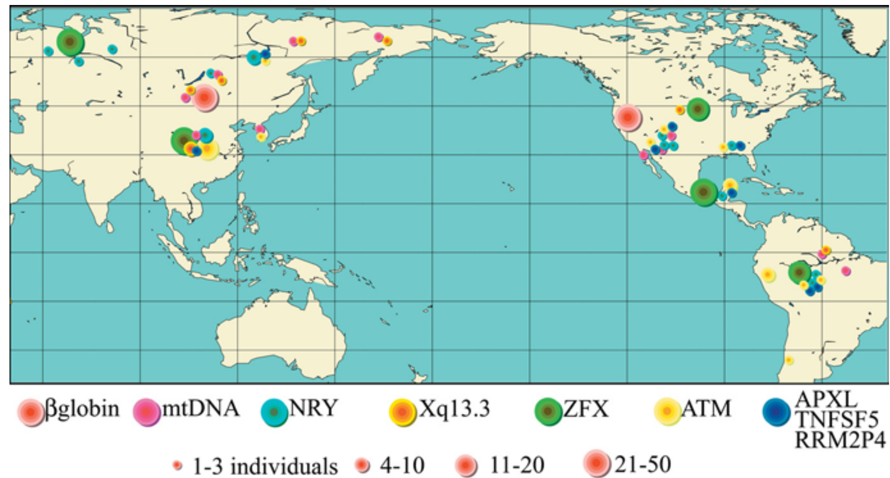
From Few to Many: New World Founded by Surprisingly Small Population

DOI: 10.1371/journal.pbio.0030227

About 14,000 years ago—a few hundred thousand years after our putative modern forebears spread out from Africa—descendants of archaic humans crossed the Bering land bridge from Siberia to North America. Several lines of evidence support this model, but that's where the consensus ends. The details remain hotly debated, focusing mostly on which Asian population migrated, when they did it, and whether they did it more than once.

Part of the challenge in reconstructing this history stems from the dynamic nature of human populations—which experience unpredictable changes in size, composition, density, and mating patterns—and the difficulty in interpreting genetic history. To get a better picture of the range of possible scenarios, scientists are using new statistical approaches that require computer simulations. Jody Hey now extends this approach in a novel method for the study of the origins of New World populations. Along with DNA analysis and computer simulations, Hey adds a new twist to an old model to reveal how the sizes of the first New World populations have changed since they were founded. His results fall in line with archeological, genetic, and linguistic evidence, pointing to a relatively recent colonization of the Americas. But they go a step further by showing that the New World was colonized by a small population with an effective size of only about 70 individuals. (The effective size refers to the number of individuals likely to contribute genes to the next generation.)

Hey's approach addresses shortcomings in population genetic studies that rely on just one gene and that assume that population sizes have been constant over time. Studying levels of DNA sequence variation at a single genomic region, or loci, can offer insight into the history of that gene, but the stochastic nature of gene evolution means that different genes have different histories. And as simplified versions of a very complex reality, population genetics models, such as the "isolation with migration" (IM) model, that aim to capture the population dynamics during the early stages of divergence or speciation have necessary limitations. The widely used IM model, for example, assumes that a founding population splits into two descendant populations that may interbreed, and incorporates a large number of parameters. But until now the IM model has required the assumption that all of the populations were constant in size, and therefore it has not been useful for assessing how descendant populations arose or changed in size.



DOI: 10.1371/journal.pbio.0030227.g001

Data from nine different regions in the human genome chart the journey of the first immigration to the New World

Hey analyzed DNA sequences from nine loci, so that the population genetic history could be found despite the variation among genes. He also added an additional parameter to the standard IM model to incorporate changes in the size of the ancestral population and of each founder population through time. The genetic data included DNA sequences from Asian and American Indian individuals. Hey varied the parameters in his model, which included founding population size, changes in population size, time of population formation (and splitting), and gene exchange between the populations, to work out the demographic scenario that best fit the available genetic data.

His analysis suggests that only about 70 individuals left their ancestral Asian population, estimated at about 9,000 individuals, to reach America 7,000 to 14,000 years ago. Archeological evidence places the earliest American inhabitants in the New World at around 14,000 years ago. Though Hey's estimates are more recent, they also indicate a high probability at this time period. Hey did not include genetic data from Eskimo-Aleut and Na Déne speakers, so the number of migrations was not addressed. But with this new approach, researchers will be able to explore this and many other questions to fill in the details of the first American immigration.

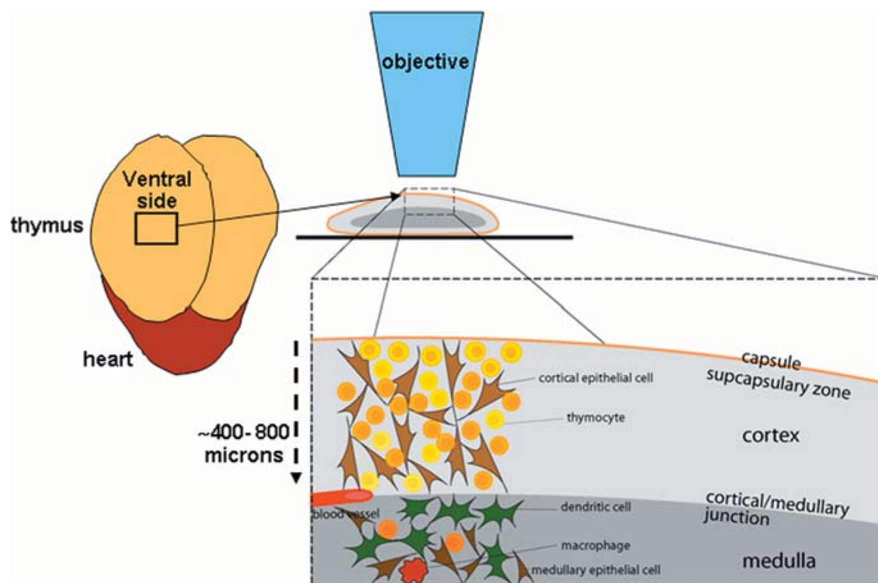
Hey J (2005) On the number of New World founders: A population genetic portrait of the peopling of the Americas. DOI: 10.1371/journal.pbio.0030193

Tracking Migrating T Cells in Real Time

DOI: 10.1371/journal.pbio.0030205

Ever since Robert Hooke startled the world with finely rendered illustrations of "minute bodies" in his 1665 book, *Micrographia*, our understanding of the microscopic world within and around us has mushroomed with each technological advance. Though imaging capability has developed light-years beyond the compound microscope that inspired Hooke's cork cell epiphany, biologists have only recently been able to observe living cells in chunks of tissue extracted from an organism—an approach that's critical for studying processes like cell differentiation and development.

Ellen Robey and her group study the mechanisms of cell differentiation and cell fate by tracking T cell development in mouse models. In a new study, Colleen Witt, Ellen Robey, and their colleagues take advantage of a recent innovation called two-photon microscopy to visualize the migration of developing T cells, or thymocytes, in intact thymuses extracted from mice. They find that after cells undergo positive selection—which



DOI: 10.1371/journal.pbio.0030205.g001

Real-time visualization of thymocytes within intact thymic lobes using two-photon microscopy

seals their fate as either helper T or killer T cells—they make a beeline for the thymus interior (called the medulla). Though it's been known that positively selected thymocytes migrate to the medulla, this study shows that migration follows a clear directional course, possibly guided by long-range signaling cues.

Because two-photon microscopy can penetrate tissue at high resolution without distorting or damaging the specimen, the authors could characterize thymocytes moving through their native tissue environment and interacting with the molecules and cells they would normally encounter. (For more on two-photon microscopy, see the Primer by David Piston [10.1371/journal.pbio.0030207] and the "Tracking the Details of an Immune Cell Rendezvous in 3-D" [DOI: 10.1371/journal.pbio.0030206].)

In the service of optimum immune defense, the mammalian adaptive immune system churns out billions of T cells a day. Precursor T cells originate in the bone marrow and migrate to the thymus, where their immune mettle is tested by a selection process that only about 1% will survive. Immature double-positive thymocytes—so-called because they have the protein markers associated with both helper (CD4) and killer (CD8) T cells—inhabit the outer thymic layer, called the cortex, while single-positive thymocytes—which have lost either the CD4 or CD8 marker following positive selection—are found in the central medulla. How a thymocyte reacts to other lymphocytes as it wends its way through the thymus determines whether it undergoes positive selection and matures into a helper or killer T cell or undergoes negative selection and programmed cell death. The signaling cues that guide this process remain obscure.

Witt et al. engineered mice with thymocytes tagged with green fluorescent protein (GFP), removed their thymic lobes for microscopic analysis when they were 4.5 to 5.5 weeks old, then observed the behavior of the glowing cells. The GFP cells in the cortex showed distinct differences in motility, morphology, and migratory behavior: low-motility cells had a spherical, nonpolar shape, moved with a modestly protruding leading edge, and sometimes paused; high-motility cells had a clear leading edge that moved in fits and starts and never paused. Once on the move, high-motility cells mostly hewed to a single direction while low motility cells often retraced their steps. Unlike the low-motility cells, the high-motility cells traveled in a linear manner through the cortex, suggesting directed migration.

Since there were so few of the fast-moving, inwardly migrating cells, the authors hypothesized that they had undergone positive selection—which they went on to confirm in transgenic mouse models. From these results, Witt et al. conclude that positive selection triggers a "rapid directional migration pattern." And because that migration corresponds to an area of the cortex that extends up to 200 microns below the outer layer of the thymus, it appears to be guided by long-range signaling cues.

As often happens in biology, close observation of a process reveals more complexity and raises more questions about the mechanics underlying it. Homing in on the source of these long-range signaling cues and characterizing the migratory patterns of the large number of slow-moving cells will go a long way toward understanding how the major components of immunity acquire their defensive chops.

Witt CM, Raychaudhuri S, Schaefer, Chakraborty AK, Robey EA (2005) Directed migration of positively selected thymocytes visualized in real time. DOI: 10.1371/journal.pbio.0030160

Human and Chimp: Can Our Genes Tell the Story of Our Divergence?

DOI: 10.1371/journal.pbio.0030202

Since humans and chimpanzees forged separate evolutionary paths some 5 million to 6 million years ago, we shed our hirsute coat and heavy brow, mastered bipedal locomotion, and acquired a knack for abstract thought while our next of kin learned to use tools, created a complex communication system, and developed the skills to construct tree-bound nests high above the forest floor. We differ by just a tad over 1% at the DNA sequence level, yet scientists predict that both species should harbor genetic footprints of our divergence.

One way to find such genetic signatures is to search for genes that reveal signs of natural selection. The assumption is that genes or genomic elements touched by natural selection will show more functionally significant molecular changes than unaffected regions. A 2003 study by Andrew Clarke et al. used this approach to identify human genes affected by positive selection—that is, selection that preserves new genetic variants—by comparing 7,645 genes from humans to their chimp and mouse equivalents. Clarke et al. identified genes in several functional categories, including olfaction and hearing, and showed that positively selected genes are more likely to contain variations (called single nucleotide polymorphisms, or SNPs) associated with genetic diseases.

In a new study, Rasmus Nielsen, Michele Cargill, and their colleagues (many of whom participated in the 2003 study)

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Young adult male chimpanzee (Photo: Frans de Waal, Emory University)

compared 13,731 known genes in humans to their equivalents in chimps to find positively selected genes in both species. Nielsen et al. also identified many genes involved in sensory perception, as well as spermatogenesis, but found the strongest evidence for positive selection in genes related to immune defense. Immunity genes, the authors explain, were likely targeted throughout mammalian evolution, while the perception and olfactory genes probably reflect primate-specific adaptations. They also found a "surprising number" of tumor-suppression and apoptosis genes.

Mutations in coding DNA can be broadly classified into two groups: a nucleotide change can cause an amino acid substitution that either alters the encoded protein (called nonsynonymous mutation) or has no effect (synonymous mutation). Nielsen et al. used a statistical method that denotes positive selection based on the ratio of nonsynonymous to synonymous mutations. Thousands of genes failed to unambiguously pass this test, leaving 8,079 for further study; those that passed were grouped into functional categories, revealing the genes related to immunity, sensory perception, and spermatogenesis. Of genes associated with specific tissues, only testis-specific genes showed evidence of positive selection. Genes expressed in the brain appear to be under selective constraints, indicating that the genetic roots of our cognitive distance from chimps lies elsewhere, perhaps in how genes are regulated or organized.

The authors followed up the chimp–human comparison by analyzing the top 50 genes from their list in a group of African- and European-Americans. The data provide further support for the conclusion that these genes have undergone positive selection.

Nielsen et al. were surprised to find so many genes involved in tumor development and control among the top 50 positively selected genes (in both primates). The factors behind this pattern are unclear, but the authors suggest that studying the genes' other functions, in immunity or spermatogenesis, may offer clues to selective pressures—and it also raises some intriguing paradoxes. It could be, for example, that the overrepresentation of genes involved in tumor suppression, spermatogenesis, and apoptosis sets up competing interests on two fronts. Apoptosis normally eliminates up to 75% of sperm cells during spermatogenesis; mutations that protect sperm cells from apoptosis may be selected for, benefiting the cell but compromising the fitness of the organism. Positive selection for apoptosis avoidance in the germ line could subsequently increase the probability of cancer in body cells—apoptosis routinely eliminates unhealthy cells—pitting the "selfish interests of a germ cell" against the organism's interest in avoiding cancer.

Future studies will have to determine whether these explanations—of an evolutionary arms race—prove plausible. We're a long way from understanding why we're so different from our closest primate cousins, but this study provides plenty of tools, and hypotheses, to mine the tiny differences in our DNA for more clues.

Nielsen R, Bustamante C, Clark AG, Glanowski S, Sackton TB, et al. (2005) A scan for positively selected genes in the genomes of humans and chimpanzees. DOI: 10.1371/journal.pbio.0030170

Uncovering the Ancient Source of Immune System Variety

DOI: 10.1371/journal.pbio.0030212

Animals with adaptive immunity have a secret for dealing with foreign invaders like viruses and bacteria—variety. Their immune systems generate a diverse array of receptors to detect the enormous number of components (antigens) that make up an invader. But with so many potential antigens, it would be difficult for the immune system to anticipate every one and thereby encode a receptor gene for each of them. Instead, the immune system employs a strategy of combinatorial diversity, recombining a few genes to give an unlimited supply of different receptors.

The portions of immune receptor genes that recombine are called V (variable), D (diversity), and J (joining) segments. The immune system randomly recombines these segments in a process called V(D)J recombination. This extraordinary reorganization is undertaken by two enzymes: RAG1 and RAG2. How this process evolved in animals is a mystery, although it has been theorized that RAG1 and RAG2 might have evolved from an ancient enzyme, called transposase, that could move or transpose gene segments. But proof of this theory for the origin of RAG's activity has remained elusive.

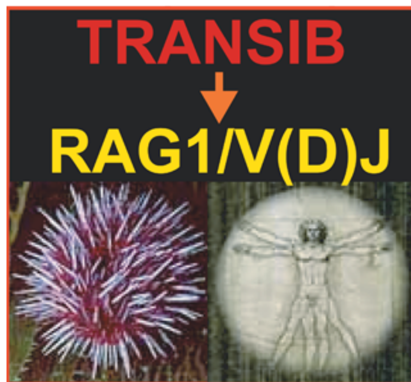
In a new study, Vladimir Kapitonov and Jerzy Jurka have found that RAG1 is similar to transposases encoded by transposons (jumping genes that encode transposases necessary for their mobility) found in both terrestrial and marine organisms: the fruit fly and malaria-carrying African mosquito and the sea urchin and hydra. These potentially ancient relatives of RAG1 are all called *Transib* transposons. The discovery of their relation to RAG1 supports the decades-old hypothesis that V(D)J recombination sprung from a transposase.

A number of different types (superfamilies) of transposons exist in nature, but no one has been able to show that RAG1 or RAG2 evolved from them. Kapitonov and Jurka took advantage of the recently discovered *Transib* superfamily of transposons to reexamine this problem. They used seven known *Transib* transposases from the fruit fly and malaria-carrying African

mosquito to search the protein database GenBank, finding that part of one *Transib* transposase, *Transib2_AG*, was 35%–38% identical to part of RAG1.

This initial relationship only suggested that RAG1 might be related to *Transib2_AG*, since the similarity between the two was only "marginally" statistically significant, leaving the possibility that it occurred by chance. To find more statistical evidence of a relationship, Kapitonov and Jurka searched for more *Transib* proteins. They found a diverse family of *Transib* transposases in various animals, including silkworm, red flour beetle, dog hookworm, soybean rust, and hydra. The authors also found that plants and vertebrates appear not to contain *Transib* proteins.

With the new proteins in tow, Kapitonov and Jurka found that a 600-amino-acid region of RAG1 was statistically similar to *Transib* transposases. This 600-amino-acid region of RAG1 forms the core region that mediates V(D)J recombination. Three important amino acids, which underlie RAG1's ability to recombine gene segments, are also conserved in *Transib* transposases. Furthermore, RAG1



DOI: 10.1371/journal.pbio.0030212 .g001

A gene involved in V(D)J recombination—which allows immune cells to recognize an unlimited number of antigens by reshuffling immune receptor gene segments—evolved from an ancient gene-transposing enzyme

and RAG2 are known to recombine V, D, and J segments by binding to specific signals in these genes (called recombination signal sequences), which appear to have been derived from the ends of *Transib* transposons. It was previously thought that both RAG1 and RAG2 likely evolved from two proteins encoded by the same transposon. However, Kapitonov and Jurka could not find any RAG2-like proteins encoded by *Transib* transposons. The authors therefore suggest that RAG2 appeared later in jawed vertebrates as a necessary component for the evolution of V(D)J recombination.

With the use of similarity searches (using computer programs to identify comparable parts of proteins and transposons), Kapitonov and Jurka have provided support for the transposon origin of V(D)J recombination. This theory was previously up for debate, as it was possible that RAG1 and RAG2 could have independently evolved to function like transposons. But the authors suggest that "these arguments can now be put to rest," as it appears RAG1 evolved from a transposon currently found in flies and other organisms. Future experiments on how *Transib* transposons work may allow further understanding into how RAG1 and RAG2 evolved and how they function in vertebrates.

Kapitonov VV, Jurka J (2005) RAG1 Core and V(D)J Recombination signal sequences were derived from *Transib* transposons. DOI: 10.1371/journal.pbio.0030181

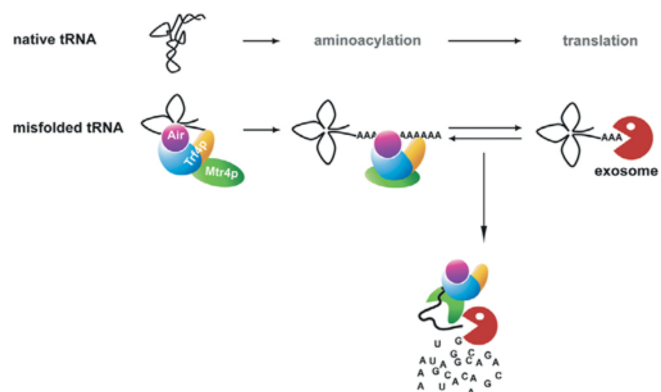
An Enzyme That Oversees RNA Quality Control

DOI: 10.1371/journal.pbio.0030191

The path from DNA to RNA to protein sounds straightforward enough, but few processes in the life of a cell could be called simple. Gene expression is a complex, highly regulated affair that involves the activity of discrete teams of molecular manipulators at key steps in the protein production pathway. As soon as transcription begins, RNA processing machinery sets to work on messenger RNA (mRNA) precursors in the nucleus, proofreading the RNA copy, stabilizing the elongating transcripts, and making sure mRNAs reach the translation machinery in the cytoplasm.

A key step in RNA processing involves the addition of a string of adenine nucleotides to the 3' end of the growing transcript, a modification called polyadenylation. (Each RNA chain has what's called a 5' end and a 3' end, which relates to the chemical polarity of the nucleotides.) In eukaryotes like yeast and humans, polyadenylation helps to stabilize the mRNA transcript and to ensure its export from the nucleus. Once in the cytoplasm, the mRNAs' poly(A) tails interact with components of the translation apparatus and facilitate protein synthesis. Polyadenylation is mediated by a class of enzymes called poly(A) polymerases (PAPs), which typically act in concert with other proteins in the cell's nucleus.

Just three years ago, a new class of PAPs was discovered that belong to the Trf4/5 family of yeast proteins and are found in the cytoplasm, rather than the nucleus, of the cell. Unlike the nuclear PAPs, in which one protein contains both catalytic activity and an RNA-binding domain, the new class relies on two or more protein subunits to carry out these tasks. It's been suggested that these enzymes stabilize specific mRNAs in the cytoplasm by extending



DOI: 10.1371/journal.pbio.0030191.g001

Model of the tRNA surveillance mechanism via polyadenylation of misfolded RNA by Trf4 complex leading to degradation by the exosome

their poly(A) tails. It's long been known that adding poly(A) tails to RNAs in prokaryotic bacteria promotes the degradation of defective RNAs, but the existence of a similar mechanism in yeast has just recently come to light. Previous genetic experiments on live yeast cells by James Anderson and collaborators suggested that when the protein Trf4p polyadenylated a particular type of abnormal transfer RNA (tRNA)—the intermediary that translates the nucleotide code into the amino acid code—the tRNA was destroyed. In a new study, Walter Keller and colleagues

investigate the biochemistry, composition, and function of Trf4p in the brewer's yeast *Saccharomyces cerevisiae*, and find evidence that Trf4p-mediated polyadenylation plays a role in RNA quality control in eukaryotes.

After showing that mutating two amino acid residues in the predicted catalytic center of Trf4p eliminates its activity, Keller and coworkers determined that the enzyme forms a PAP complex with three other proteins (including two putative RNA-binding proteins, Air1p and Air2p, and a putative RNA-unwinding enzyme called Mtr4p that most likely functions to unwind structured regions in the RNAs). They also showed that the Trf4p complex selectively targets and successfully polyadenylated only tRNA molecules that were either lacking the chemical modifications required for normal folding or that were misfolded by mutation. Polyadenylation appears to tag the aberrant RNA as damaged goods, signaling the cell's nuclear molecule-degradation complex, the exosome, to initiate destruction.

Keller and colleagues propose that the Trf4p complex recognizes structural defects in RNA, prompting the Trf4p subunit to add poly(A) tails to the RNA, which initiates RNA degradation. In this model, Trf4p, along with either Air1p or Air2p, interacts with the RNA enzyme Mtr4p, which physically connects the tRNA-Trf4-PAP complex to the exosome. These results suggest that Trf4-PAP monitors the quality of tRNAs by detecting misfolded RNAs and engineering their destruction before they can gum up the works of protein assembly.

That the polyadenylation pathway for discarding defective tRNA appears in both bacteria and yeast suggests that this quality-control mechanism represents the ancient role for polyadenylation, the authors propose; the stabilization function of adding poly(A) tails may have arisen as eukaryotes evolved a nucleus and other organelles. Whether this notion or the model described here proves correct remains to be seen, and the authors outline a number of avenues for further study. Determining the structure of RNA–protein complexes, for example, and their binding properties and interactions, the authors argue, should elucidate the mechanism by which this RNA surveillance complex operates and what features of its RNA substrates it recognizes.

Vaňáčová S, Wolf J, Martin G, Blank D, Dettwiler S, et al. (2005) A new yeast poly(A) polymerase complex involved in RNA quality control. DOI: 10.1371/journal.pbio.0030189

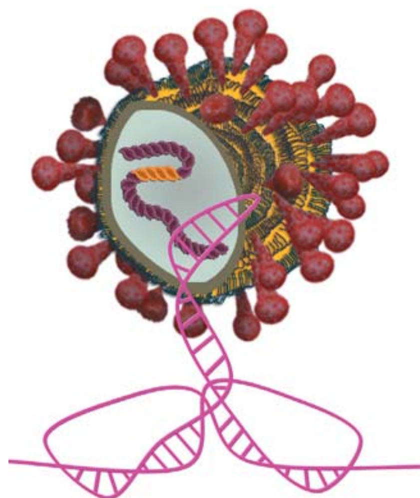
New Frameshifting Pseudoknot Found in SARS Virus

DOI: 10.1371/journal.pbio.0030199

Viruses, small organisms that hijack our cellular machinery to replicate their genomes and make new viruses, constantly threaten human health. Not only are we unable to control infections caused by old enemies such as the cold virus, but we are also continually challenged by new enemies, like the coronavirus that causes severe acute respiratory syndrome (SARS-CoV). Vaccines provide one way to deal with viruses, but subtle differences between how host and viral proteins are made may also provide targets for new antiviral therapeutics.

SARS, a life-threatening respiratory illness, first appeared in late 2002. By February 2003, Guangdong Province, China, was in the grip of a SARS epidemic, and public-health officials were predicting that millions of people might become infected. The rapid implementation of effective containment efforts averted this new threat to human health, and, in the end, only 8,098 people became ill. SARS-CoV, a single-stranded RNA virus, was isolated in March 2003 and its genome sequenced by May 2003. Since then, researchers have intensively studied the virus, hoping to identify targets for antiviral therapeutics. Jonathan Dinman and colleagues now describe a new RNA structural motif in the SARS-CoV genome that may provide such a target.

During protein synthesis, molecular machines called ribosomes move along mRNA molecules, translating nucleotide triplets into amino acids. In human cells, the ribosomes usually hook onto the start of an mRNA and decode each triplet in turn. However, viral mRNAs often contain special signals that tell the ribosomes to change register or "frameshift." This allows viruses to coordinate gene expression from overlapping coding sequences, and it ensures that the correct ratios of enzymatic and structural proteins are made.



DOI: 10.1371/journal.pbio.0030199.g001

A three-stemmed pseudoknot in SARS messenger RNAs could provide a target for antiviral therapeutics (Image: Jonathan Dinman and the National Institute of Allergy and Infectious Diseases)

ORF1a and ORF1b are overlapping, out-of-frame coding sequences within the SARS-CoV genome. Each encodes a polyprotein—a large protein that is cleaved into smaller, functional proteins. Polyprotein 1a is translated directly from ORF1a; the fused polyprotein 1a/1b is produced by programmed −1 ribosomal frameshifting in which the ribosome slips back one nucleotide at a special signal within the mRNA. Like other frameshift signals, the SARS-CoV signal contains a pseudoknot, a stable mRNA structure. Pseudoknots generally contain two stems, in which complementary nucleotides form double-stranded RNA, and two or three loops of unpaired nucleotides. Because the mRNA strand passes over and behind itself to form the stems, the whole structure looks like a small knot of the kind that would unravel if its two ends were pulled.

Unexpectedly, a computational analysis undertaken by Dinman and his colleagues reveals that the pseudoknot in the SARS-CoV frameshift signal contains three stems. The researchers provide further evidence for this novel structure by finding potential three-stemmed pseudoknots in the frameshift signals of other coronaviruses and by doing biochemical and structural NMR studies on the SARS-CoV signal. They also show that the SARS-CoV frameshift signal behaves like other viral frameshift signals in several frameshifting assays, and their mutagenesis studies indicate that specific sequences and structures within

stem 2 of the pseudoknot are needed for efficient frameshifting.

The exact role of the extra stem in the SARS-CoV frameshifting signal remains to be determined, but the researchers speculate that it could help to regulate the exact ratio of polyprotein 1a to 1a/1b. The current results also suggest that the three stems may fold back on one another to from a complex globular RNA structure. The elucidation of this structure by high-resolution NMR, the researchers say, should facilitate the rational development of therapeutic agents designed to interfere with SARS-CoV programmed −1 ribosomal frameshifting and should also increase our understanding of how pseudoknots stimulate frameshifting.

Plant EP, Pérez-Alvarado GC, Jacobs JL, Mukhopadhyay B, Hennig M, et al. (2005) A three-stemmed mRNA pseudoknot in the SARS coronavirus frameshift signal. DOI: 10.1371/journal.pbio.0030172

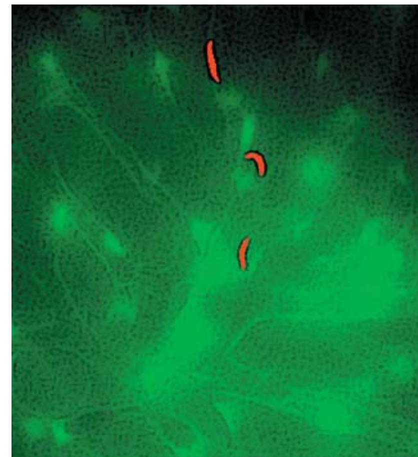
Tracking a Killer: In Vivo Microscopy Reveals Details on the Life Cycle of Malarial Parasites

DOI: 10.1371/journal.pbio.0030215

In a perfect world, anyone frequenting the habitat of malaria-carrying mosquitoes would sleep beneath a mosquito net, take prophylactic drugs, and maybe even quaff quinine-laced tonic water. But life is rarely perfect and even though governments and the World Health Organization have combined such prevention measures with widespread efforts to eradicate malaria-carrying mosquitoes, the disease kills more than a million people each year.

The microbes that cause the disease, tiny parasites of the genus *Plasmodium*, have a complex life cycle that involves several distinct phases and habitats. When a person is bitten by an infected mosquito, highly mobile *Plasmodium* cells (called sporozoites) migrate from the skin into the bloodstream, which carries them to the liver, where they set up shop in liver cells (called hepatocytes) and multiply asexually as merozoites. Eventually the parasites leave the liver and reenter the bloodstream, where they invade red blood cells, multiply again, and differentiate into new merozoites. Ultimately, a red blood cell will become so chock-full of merozoites it bursts, releasing more merozoites to infect other red blood cells. Some of these merozoites will differentiate into male and female sex cells (called gametocytes) that hitchhike along with red blood cells when a new mosquito takes a blood meal from an infected person. The gametocytes then breed within the mosquito and produce sporozoites, which reside in the insect's gut—and then the cycle begins all over again.

Although much is already known about the *Plasmodium* life cycle, many details—including the discrete steps that facilitate sporozoites' invasion of the liver—have escaped direct observation until now. In a new study, Ute Frevert et al. literally take a closer look at this process with intravital microscopy—which allows direct observation of cell movement in a living animal—to see how the parasites gain entry into the liver.



DOI: 10.1371/journal.pbio.0030215.g001

Tagged with red fluorescent protein, the malarial parasites in the sporozoite stage can be seen migrating along the sinusoids in a mouse liver

To visualize this process, the authors used genetically engineered *Plasmodium* parasites to express fluorescent tags. They introduced these fluorescent parasites to mice and rats the natural way—through mosquito bites—and then watched for the arrival of the parasites in the livers of the test animals. They observed that parasites were carried into the liver by the bloodstream, and then attached to the sinusoidal walls (sinusoids take the place of capillaries in the liver). They watched as the parasites crawled along the interior of the sinusoids—sometimes against the direction of blood flow—until they reached a specialized cell called a Kupffer cell. These star-shaped cells line the liver sinusoids and clean the blood of particulate debris and dead blood cells. But they also, apparently, serve as the parasites' portal of entry into the liver.

Earlier work had suggested that sporozoites might use Kupffer cells to access the liver, but Frevert et al. watched sporozoites traverse Kupffer cells to reach the liver interior. They observed some interesting details in this process: as sporozoites enter a Kupffer cell, they first pause, then undergo a slow constriction as they insinuate their way through the cell (rather like a napkin being drawn through a napkin ring). Sporozoites traverse the Kupffer cell at a speed much slower than they could crawl, indicating that this traversal involves more than mere parasite locomotion. Upon exiting the Kupffer cell on the other side, the sporozoites wreaked havoc in the liver, leaving a path of destruction and dead cells behind them as they moved through several consecutive hepatocytes before finally settling down in one to begin reproducing.

Frevert et al. had to make multiple attempts and track several different sporozoites at each stage in order to gain a comprehensive picture of this part of the parasite's life cycle. But thanks to the visualization advantages provided by using the fluorescent parasites and intravital microscopy, the authors show that it is now possible to directly observe events in the *Plasmodium* life cycle that had only been inferred before.

Frevert U, Engelmann S, Zougbédé S, Stange J, Ng B, et al. (2005) Intravital observation of *Plasmodium* berghei sporozoite infection of the liver. DOI: 10.1371/journal.pbio.0030192

A Dying Cell's Last Hurrah: Flagging the Remnants of a Fatal Infection

DOI: 10.1371/journal.pbio.0030197

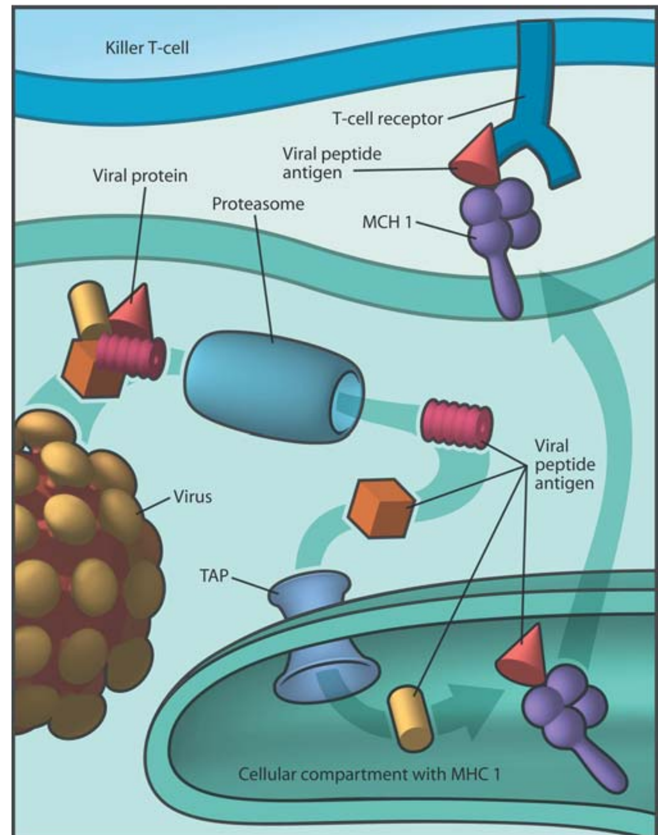
For a cell, suicide is a perfectly acceptable response to stress. Programmed cell death, or apoptosis, is a normal part of development: it occurs 131 times over the course of worm development, for example, and throughout the life of an organism to replenish worn out cells in a variety of tissues. Disease and infection also trigger apoptosis. The dying cell shrinks into a membrane-bound package that attracts the attention of phagocytes, which quickly ingest and degrade the cell's contents. Dendritic cells, specialized phagocytes, patrol the body, capturing molecules (or antigens) expressed by a host of pathogens, parasites, environmental substances, and apoptotic cells, and present their findings to the helper and killer T cells that launch an immune response.

Before dendritic cells can sound the immune alarm, they must capture antigens, chop them into peptide fragments, and bind the antigenic peptides to molecules of the major histocompatibility complex (MHC). Though dendritic cells can present to helper or killer T cells, it had been thought that the MHC I–bound antigens (recognized by killer T cells) must first enter the dendritic cell's cytosol and undergo proteasome degradation. It was also thought that antigenic peptides needed the transporter activity of TAP (transporter associated with antigen processing) proteins to escort them into the cellular compartment where the MHC I molecules reside.

A new study by Nathalie Blachère, Robert Darnell, and Matthew Albert lends support to an emerging view that antigen processing may be more flexible and suggests that dendritic cells can generate MHC I peptides that activate killer T cells through cross-presentation. (The phenomenon is called cross-presentation because the pathway crosses established rules of MHC I antigen presentation.) Working with a mouse model to study killer T cell activation in response to influenza antigens derived from apoptotic cells, the authors show that two distinct pathways mediate antigen processing. In the first pathway, antigen processing in dendritic cells depends on TAP, following the established model. But in the second pathway, antigens processed by a dying, infected cell can bypass the dendritic cells' degradation machinery yet still permit generation of MHC I/peptide complexes that can engage killer T cells.

T cells can recognize millions of different antigens because each T cell is outfitted with a unique T cell antigen receptor that recognizes a unique MHC/peptide complex. In this study, the authors used the activation of influenza-reactive T cells as evidence of antigen presentation. To test the hypothesis that dying cells can process antigen for cross-presentation, Blachère et al. limited the dendritic cells' ability to process antigen by using cells extracted from the bone marrow of mice lacking functional TAP proteins. If the cells could provoke a T cell response, then antigen-specific MHC I/peptide complexes would have to arise through an alternate pathway.

When TAP-deficient dendritic cells were directly infected with influenza, they could not elicit a T cell response. But when infected dying cells were internalized by the TAP-deficient dendritic cells, the dendritic cells were able to cross-present influenza antigen and activate the T cells. To investigate the mechanism of cross-presentation, the authors generated cells with deficient proteasomes that could not degrade viral proteins, and also used another cell line inhibited in TAP activity. When either of these cell types were infected with influenza, caused to undergo apoptosis, and engulfed by dendritic cells, it became clear that the dendritic cells needed functional TAP to successfully present the influenza antigen to T cells. The authors then went on to validate the operation of these pathways in vivo.



DOI: 10.1371/journal.pbio.0030197.g001

Antigenic proteins in the cytosol are degraded by the proteasome and transported by TAP into the MHC1 compartment for MHC1 loading (Image: Giovanni Maki)

Altogether, these results point to two MHC I pathways: one relies on transporter activity in the dendritic cell; the other allows dendritic cells to capture antigens already processed in the dying cell. Though the details of the cross-presentation pathway remain unclear, the conditions that give rise to it are not: when dendritic cells are directly infected, they mediate antigen processing. When dendritic cells ingest already infected, apoptotic cells, the dying cells deliver processed antigen and set the stage for cross-presentation. It's possible that stressful conditions, like an influenza infection, trigger this cross-presentation pathway, allowing the dying cell to alert the immune system to a pathogenic agent that requires immediate action.

Blachère NE, Darnell RB, Albert ML (2005) Apoptotic cells deliver processed antigen to dendritic cells for cross-presentation. DOI: 10.1371/journal.pbio.0030185

Bacterial SOS May Be the Key to Combating Antibiotic Resistance

DOI: 10.1371/journal.pbio.0030221

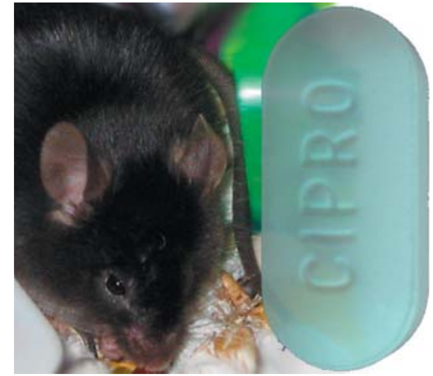
The development of antibiotic drugs changed the face of clinical medicine forever, and, for a short while at least, it seemed that a perfect cure for bacterial infections had been discovered. But widespread overuse and misuse of antibiotics over the last several decades has proved the Achilles heel of this once seemingly invincible class of drugs and has fostered bacterial resistance to conventional antibiotic therapies. New drugs are continually being developed to replace those crippled by resistance, but despite scientists' best efforts, new "superbugs" are evolving faster than the drugs required to control them.

For some time now, drug resistance has been considered a consequence of errors (called mutations) that accumulate spontaneously during replication of the bacterial genome. In many cases those mutations are either inconsequential or harmful to the bacteria, but on rare occasion, they provide an accidental benefit: resistance to the drugs that kill them. Because the mutations were assumed to be spontaneous, there was no obvious way to prevent them and thus antibiotic resistance appeared inevitable. But some researchers are taking a pro-active approach by testing the assumptions about how mutations are made. For one group of bacterial researchers, this approach may have paid off.

Chemists at the Scripps Research Institute and the University of Wisconsin have uncovered evidence that spontaneous mutations are not the only way in which bacteria acquire resistance to antibiotics. It appears that the bacteria, rather than passively waiting around for a lucky break, may play an active role in their own evolution. The key is in the way antibiotics interact with their bacterial targets. Quinolone antibiotics are a relatively new class of antibiotics that work by interfering with proteins called topoisomerases, which assist DNA replication by loosening tightly wound DNA and making it accessible. In order to do this, the topoisomerase must break the DNA strands and fill in the gap with a temporary protein bridge. Under normal circumstances, the bridge is removed and the DNA is reconnected after the topoisomerase has done its job, but quinolones bind to this protein bridge and prevent the DNA from resealing. The freed double-strand ends signal that DNA damage has occurred and activate the cell's repair pathway.

According to Ryan Cirz et al., DNA damage, induced by antibiotics or other stressors, sets off a bacterium's emergency repair mechanism: the SOS DNA damage response. Under normal conditions, the genes are turned off by a special repressor protein called LexA. In response to the damaged DNA, the LexA repressor is cleaved and no longer inhibits transcription of the SOS response genes. Cirz et al. propose that antibiotic-mediated DNA damage generates a reduction in the concentration of LexA that is sufficient to increase the expression of three nonessential DNA polymerases shown to be required for mutation: Pol II (encoded by the gene *polB*), Pol IV (encoded by *dinB*), and Pol V(encoded by *umuD* and *umuC*). Together these polymerases promote DNA repair—and cause mutations in bacterial DNA that can lead to antibiotic resistance.

This suggests that quinolone antibiotics (and other antibiotics that cause similar kinds of DNA damage) may increase the likelihood that bacteria will evolve resistance and that new generations of drugs will have little chance of succeeding where today's drugs have failed. But all hope is not lost. After showing that the evolution of quinolone resistance depends on activating the SOS response genes gated by LexA, Cirz et al. go on to demonstrate that blocking LexA cleavage, in vitro and in a mouse model, prevents mutation and results in bacteria that are unable to evolve antibiotic resistance. Thus, developing novel therapeutic agents that target LexA or the associated SOS pathway may prove a promising strategy for controlling the spread of the superbugs.



DOI: 10.1371/journal.pbio.0030221.g001

Inhibiting a protein involved in DNA repair in bacteria might prevent mutations that promote antibiotic resistance, prolonging the effectiveness of ciprofloxacin and other quinolone antibiotics

Cirz RT, Chin JK, Andes DR, de Crécy-Lagard V, Craig WA, et al. (2005) Inhibition of mutation and combating the evolution of antibiotic resistance. DOI: 10.1371/journal.pbio.0030176

Tracking the Details of an Immune Cell Rendezvous in 3-D

DOI: 10.1371/journal.pbio.0030206

If you stopped to consider the potentially pathogenic encounters waiting outside your door on any given day, you might never leave home. Then you would just have to contend with the billions of microbes reproducing on your toothbrush and kitchen sponge. Of course, most of us safely navigate our microbe-filled world thanks to an immune system that manufactures billions of lymphocytes a day, in case any of those microbes proves malicious.

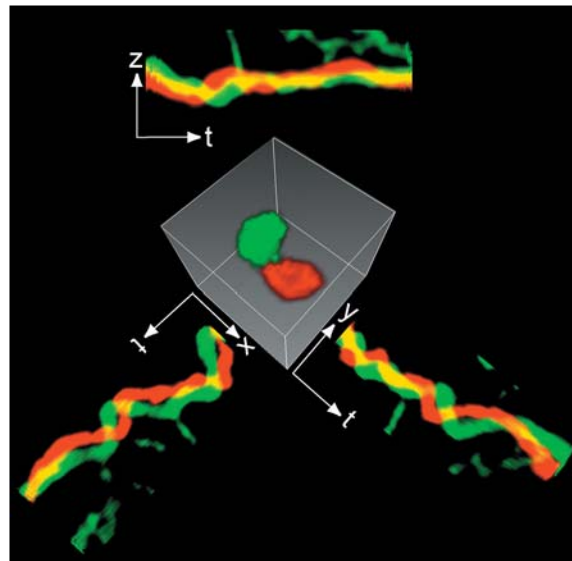
Lymphocytes arise in the bone marrow, where B lymphocytes mature; T lymphocyte precursors migrate to the thymus to mature. Both T and B cells then travel to distinct regions in the spleen and lymph nodes—B cells amassing in follicles and T cells in T zones—in search of alien antigens. After encounters with antigen, B and T lymphocytes migrate to the edges of their respective zones and compare notes. These B cell–helper T cell interactions are essential for an effective B cell–mediated antibody response. One would not expect such crucial interactions to be left to chance alone, but evidence of directed migration has not been generated—until now.

In a new study, Takaharu Okada and Jason Cyster at the University of California at San Francisco together with Mark Miller and Mike Cahalan at the University of California at Irvine and several colleagues used a groundbreaking technology called two-photon microscopy to visually inspect intact lymph nodes extracted from mice to investigate this lymphocyte rendezvous.

They discovered a combination of random and directed behaviors: antigen-engaged B cells move randomly along the follicle outskirts, then undergo directed migration near the follicle/T-zone border as they home in on their helper T counterparts. (For more on two-photon microscopy, see the Primer by David Piston [10.1371/journal.pbio.0030207] and "Tracking Migrating T Cells in Real Time" [DOI: 10.1371/journal.pbio.0030205].)

Once a B cell recognizes an antigen via immunoglobulin receptors on its surface, it will not proliferate, differentiate into a plasma cell, and generate mass quantities of antibodies without the go-ahead from a helper T cell. To investigate the dynamics of this process, Okada, Miller, and colleagues transferred fluorescently labeled HEL-specific transgenic B cells, or Ig-tg B cells (engineered to secrete antibodies against the model antigen, hen egg lysozome [HEL]), and nonaltered B cells into mice with identical genetic backgrounds. An hour after HEL injections, lymph nodes were removed from the mice for microscopic analysis. The Ig-tg B cells were "fully occupied" by HEL antigen and—unlike the naïve (unengaged) non-Ig-tg cells—had begun to aggregate along the edge of the follicles.

After antigen binding, the Ig-tg cells grew sluggish compared to naïve cells, then headed for the B-zone/T-zone (B/T) border. Half of the primed cells reached the B/T boundary, compared to 20% of the naïve cells, and did so by (mostly) taking a path that was closer to a straight line—a sign of directed migration. Ratios of path length plotted against displacement from the path show that antigen-engaged B cells tacked toward the boundary when they got within about 140 microns of it.



DOI: 10.1371/journal.pbio.0030206.g001

Two-photon microscopy reveals the three-dimensional dynamics of B cell (red) and T cell (green) conjugate within lymph tissue in real time

The authors go on to show that antigen-engaged B cells need the chemokine receptor CCR7 to follow directions to the T zone—which contains an abundant supply of CCR7's signaling protein, or ligand—CCL21. Besides concentrating in the T zone, CCL21 also showed up in follicles, in an increasing gradient from the follicle periphery to the boundary. Okada, Miller, and colleagues studied interactions between antigen-engaged B cells and activated helper T cells in a transgenic mouse model and found that only B and T cells with cognate antigens formed stable pairs, which moved at the B cells' discretion.

Based on these findings, the authors conclude that once antigen-engaged, B cells follow the long-range chemokine gradient to the B/T boundary. After arrival at the boundary the B cells can undergo multiple, even polygamous, contacts with T cells—which might facilitate optimal pairings—before B cell proliferation and antibody production begins. Whether promiscuous and monogamous liaisons produce different B cell reactions is unclear. And though CCR7 helps B cells find the border, it's not clear what keeps them there. But thanks to the T lymphocyte–B lymphocyte dynamics outlined here, immunologists have plenty of avenues for exploring these questions to further elucidate the complex interactions underlying an effective antibody attack.

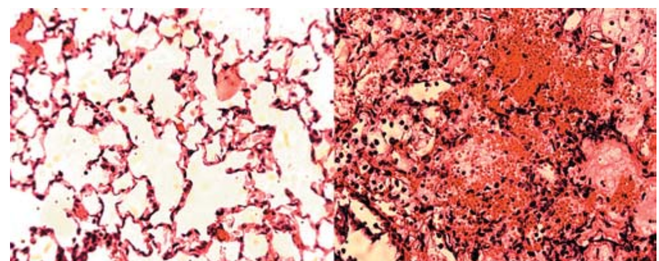
Okada T, Miller MJ, Parker I, Krummel MF, Neighbors M, et al. (2005) Antigen-engaged B cells undergo chemotaxis toward the T zone and form motile conjugates with helper T cells. DOI: 10.1371/journal.pbio.0030150

Hypoxia to the Rescue: When Oxygen Therapies Backfire

DOI: 10.1371/journal.pbio.0030211

If a novel oxygen-producing metabolic pathway hadn't evolved in ancient microbes over 3 billion years ago, it's unclear whether humans and other oxygen-dependent species would have either. But evolve we did, adapted to having just the right level of oxygen coursing through our blood—too little oxygen (hypoxia) causes headaches, nausea, and eventually death. Patients with acute respiratory distress syndrome (ARDS) and other serious lung injuries routinely receive oxygenation therapy to facilitate oxygen delivery to deprived tissues. But too much oxygen (hyperoxia) kills, too. Hyperoxia produces free radicals, causing oxidative damage to cells and tissues by disrupting cellular components. And recent evidence suggests that oxygenation therapy might produce dangerous side effects in patients with ARDS who also have severe pulmonary inflammation.

In a new study, Manfred Thiel et al., in a team led by Michail Sitkovsky, test the hypothesis that oxygenation weakens a tissue-protecting mechanism triggered by hypoxia. Working with gene-altered mice, the team of immunologists, pathologists, and biochemists finds evidence that clinical oxygenation treatments


DOI: 10.1371/journal.pbio.0030211.g001

Lung tissue under ambient oxygen levels (left) and under 100% oxygen (right), which exacerbates acute inflammatory lung injury

could aggravate lung injury by inhibiting this protective pathway. But this protective pathway could potentially be restored, they argue, by artificially activating the inhibited pathway with therapeutic activators. Their results have important implications for how patients with ARDS and other serious lung diseases should be treated.

Hypoxia triggers a signaling pathway mediated by an adenosine receptor (called $A_{2A}R$) that arrests inflammation and tissue damage. It's thought that this same hypoxia-driven pathway protects the lungs from the toxic effects of overactive immune cells called neutrophils. Using a mouse model of acute lung injury induced by bacterial infection, Thiel et al. exposed one group of mice to 100% oxygen, mimicking therapeutic oxygenation, and left another group at normal ambient levels (21% oxygen). Five times more mice died after receiving 100% oxygen than died breathing normal oxygen levels. Mice given 60% oxygen—considered clinically safe—got worse, but didn't die.

To test the hypothesis that oxygen was precipitating these drastic results by exacerbating tissue inflammation, the authors analyzed the neutrophil-mediated immune response. Establishing a correlation between high neutrophil count and increased capillary leakage—indicated by the protein concentrations recovered from the alveolar space, which mediates gas exchange—Thiel et al. confirmed that overactive neutrophils promote lung injury. When otherwise healthy mice were subjected to lung infection and treated with hypoxia (10% oxygen), after 48 hours 90% of the mice showed several signs of improvement associated with an inhibition of neutrophil-mediated inflammation.

Thiel et al. went on to show that the adenosine receptor pathway was involved in the oxygen-dependent inflammation. By isolating neutrophils from mice with inflamed lungs and exposing them to high concentrations of a molecule that activates the adenosine receptor, they triggered increased levels of both $A_{2A}R$ and cAMP, a molecule that inhibits inflammation. No such increases were seen in mutant mice lacking functional $A_{2A}R$ proteins.

Hypoxia protects against lung damage, the authors conclude, by working through the $A_{2A}R$ signaling pathway to control inflammation. Above-normal oxygen levels interrupt this anti-inflammatory pathway, paving the way for further lung injury. Administering a molecule that jump-starts $A_{2A}R$ signaling artificially also significantly reduced the pathological side effects of oxygenation. These results may help explain why some patients with ARDS die following oxygenation therapy. And by identifying the mechanism that is disrupted by oxygenation—$A_{2A}R$ signaling—this study suggests that therapies aimed at activating the anti-inflammatory $A_{2A}R$ pathway may allow patients to receive the benefits of oxygenation therapy without succumbing to its toxic effects.

Thiel M, Chouker A, Ohta A, Jackson E, Caldwell C, et al. (2005) Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. DOI: 10.1371/journal.pbio.0030174

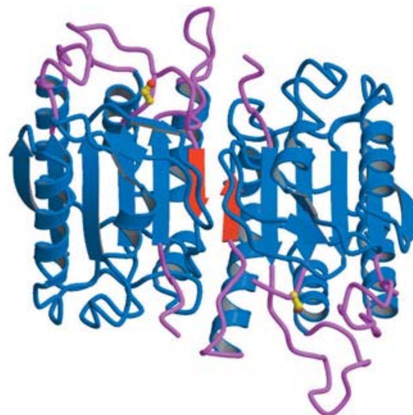
Proximity- or Conformation-Induced Caspase Activation?

DOI: 10.1371/journal.pbio.0030198

In 1972, John Kerr, Andrew Wyllie, and Alistair Currie introduced the word "apoptosis" (from the Greek for apples falling off trees) to describe a special form of cell death. Apoptosis, or programmed cell death, is a normal physiological process in which specific signals trigger cells to self-destruct in a carefully choreographed manner. Without apoptosis, we would have paddles for hands, rather than individual fingers. And without apoptosis, we would be at much greater risk of developing cancer, since apoptotic mechanisms destroy cells that have taken the first steps toward tumor formation.

Cells apoptose when the positive signals needed for their survival are withdrawn or when negative signals tell the cell to commit suicide. A cell undergoing apoptosis shrinks and develops "blebs"—small bubbles—on its surface, its mitochondria break down, and its genomic DNA breaks into fragments. Finally, the dead cell is engulfed by phagocytic cells, thus avoiding any inflammation in surrounding tissues.

Apoptosis can be triggered by external and internal signals, but in both the extrinsic and intrinsic pathway of apoptosis, once a signal has been received, the next molecular step is caspase activation. These proteases, which cleave proteins at specific sites, fall into two classes. Initiator caspases activate themselves before proteolytically activating the effector (or executioner) caspases, which degrade numerous cellular proteins, thus causing cell death. All the caspases are made as inactive enzymes (zymogens); they are much too dangerous to be stored as active enzymes.



DOI: 10.1371/journal.pbio.0030198.g001

Experiments with artificially joined molecules of caspase 9—which normally exists as monomers (above)—suggest that caspase 9 can't activate cell death pathways by dimerization alone

Yigong Shi and colleagues are studying the mysterious process of initiator caspase activation—the molecular mechanism of effector caspase activation is fairly well understood. The autocatalytic activation of caspase-9, an initiator caspase in the intrinsic pathway of apoptosis, is mediated by the assembly of the apoptosome, a heptameric complex containing Apaf-1 (apoptotic protease activating factor 1) and cytochrome c. Caspase-9 activation is currently explained by the induced proximity model in which the apoptosome, by increasing the local concentration of caspase-9, promotes its homodimerization (the formation of a complex containing two caspase-9 molecules) and subsequent autoactivation.

To test this model, Shi and coworkers engineered caspase-9 so that it exists in the cell all the time as a homodimer—wild-type caspase-9 usually exists as monomer—and then examined its catalytic activity. They found that

although the engineered, dimeric caspase-9 was more active in in vitro assays and induced more cell death when expressed in cells than the wild-type enzyme, its activity was only a fraction of that of Apaf-1-activated wild-type caspase-9. Furthermore, its activity was not stimulated by Apaf-1, unlike that of the wild-type enzyme. Importantly, they also show that the crystal structure of their engineered caspase-9 closely resembled that of wild-type caspase-9, indicating that the changes they made did not cause any significant changes in the protein.

Overall, their results led the researchers to suggest that the dimerization of caspase-9 may be qualitatively different from the Apaf-1-mediated activation of caspase-9, and that dimerization may not be the major mechanism behind the activation of caspase-9. Instead, they suggest that a shape (conformational) change is induced in caspase-9 when it binds to the apoptosome and that this change drives its activation. This "induced conformation" model provides an alternative model to the induced proximity model for initiator caspase activation, but, as the researchers note, the two models need not be mutually exclusive. Indeed, only the determination of high-resolution structure of the apoptosome will unravel exactly how caspase-9 and other initiator caspases are activated.

Chao Y, Shiozaki EN, Srinivasula SM, Rigotti DJ, Fairman R, et al. (2005) Engineering a dimeric caspase-9: A re-evaluation of the induced proximity model for caspase activation. DOI: 10.1371/journal.pbio.0030183

Fly Movie Theater Reveals Secrets of How Insects See the World

DOI: 10.1371/journal.pbio.0030209

Seeing the world pass by as you're moving is a complex feat. In flies, images moving across the eye—so-called optic flow—provide sensory information to neurons to guide behaviors like avoiding obstacles and chasing down mates. This maneuverability, combined with a fast image processing speed and the ease of examining fly neurons, has made flies a popular model for dissecting how this occurs. But since much of what we know about how neurons process visual information comes from static situations—for example, placing a fly in a fixed position and presenting it with moving images—the question of how these processes naturally occur is largely unexplored.

In a new study, a German and a Dutch group headed by Martin Egelhaaf and Roland Kern and by Hans van Hateren, respectively, joined forces to take the analysis of optic flow in the blowfly one step closer to the natural situation. To do this, they used a "panoramic virtual reality stimulator" to show a fly in the laboratory what it would see while flying in nature. The authors combined this with measurements of the response of a motion-sensitive blowfly neuron, called the horizontal system equatorial (HSE) cell.

The traditional model holds that the HSE cell extracts information about the motion of a fly from optic flow. Previous work, which involved recording the responses of the HSE cell to simple visual stimuli (measured as a change of electrical potential of the neuron), suggested that HSE responds only to rotations of the visual world. However, the use of more natural visual stimuli suggests that some functions of the HSE cell may have been missed.

DOI: 10.1371/journal.pbio.0030209 .g001

HSE neurons in the blowfly allow the fly to extract visual information about the depth structure of the world during flight

How HSE cells respond cannot be recorded in freely moving animals due to technical difficulties. To get around this problem, the Dutch group recorded the free flight of blowflies, including their characteristic head and body movements. The visual stimuli during this behavior were then reconstructed in computer-generated simulations and played back by the German group to flies in the panoramic virtual reality stimulator. Since this was done in the laboratory, the authors could record the responses of the HSE cell as the flies watched this movie.

Normal blowfly flight style involves a combination of saccadic (jerky) turns, where the head rotates at high velocity, and periods of forward motion accompanied by a constant gaze. During a playback of ten different versions of this behavior, the authors did not see a positive change in potential of the HSE cell during saccadic turns, as might be expected from previous conventional recordings. Instead, the HSE cell was depolarized by optic flow between saccades—when the fly's head was not rotating.

This surprising result suggests that blowflies may gather useful visual information about the world from translational (movement without rotation) optic flow—when their heads are not rotating and their gaze is fixed. In fact, the authors found that the blowfly's flight strategy allows information to be extracted from translational optic flow under situations where optic flow from rotation might otherwise dominate. Thus, HSE cells may not only encode information from dominant rotations of the fly itself, but allow the fly to extract "behaviorally relevant information" about the depth structure of the world. However, as the authors point out, it is not yet known whether the blowfly's nervous system can garner rotational and translation information from the combined output of HSE cells.

Nevertheless, through the use of a novel method to play back natural flight behaviors, the authors have been able to discern a new function for a well-studied motion-sensitive neuron—a function that appears to emerge from the blowfly's own behavior. Future experiments can now begin to explore how the fly uses the information generated by this new function.

Kern R, van Hateren JH, Michaelis C, Lindemann JP, Egelhaaf M (2005) Function of a fly motion-sensitive neuron matches eye movements during free flight. DOI: 10.1371/journal.pbio.0030171

Switching Signals in the Brain

DOI: 10.1371/journal.pbio.0030210

The brain is arguably the most complex computing machine on the planet, with billions of individual neurons that process the input they receive from sensors in the body and from other neurons. The composition of channels that regulate electrical conductance across the neuronal membrane is a key determinant of the exact pattern of a neuron's response to the stimuli it receives. Bursting—brief epochs of rapid firing—is one such pattern, while single spiking, with longer intervals between firing, is another.

In a deep-seated part of the cerebral cortex, the hippocampus receives input from and sends output to widespread regions of the brain; information processing in the hippocampus is thought to underlie aspects of memory. The output of the hippocampus is handled by a subregion, the subiculum, that exhibits response modes that correspond to either bursting or single-spike response patterns. In this issue, Don Cooper, Sungkwon Chung, and Nelson Spruston show that the bursting–spiking switch is controlled by a new and surprisingly simple mechanism, prolonged inactivation (shutting off) of channels that conduct sodium ions through the membrane.

Working with rats, the authors measured activity from bursting subicular neurons in vivo, and observed frequent transitions from bursting to single spiking. Bursting tended to occur after long silent periods, while single spiking predominated after short intervals. To determine what drove this change from bursting to single spiking, they examined electric activity in brain slices. The switch was strongest when neurons were stimulated at frequencies between 1 and 10 Hertz, which suggested an inactivation and recovery process, perhaps mediated by prolonged inactivation of a small number of sodium channels. To test this hypothesis, the authors applied a very low concentration of tetrodotoxin, a neurotoxin from puffer fish that specifically blocks sodium channels. Indeed, blocking 16% of the channels with the toxin induced a switch from bursting to single spiking even at very low frequency stimulation, when subicular neurons normally maintain their bursting pattern.

Output mode switching by the sustained inactivation of sodium channels is a novel mechanism for controlling the dynamics of neural networks. While the functional significance of the switch remains unexplored, the authors point out that bursting is known to effectively activate target structures. It may be that switching from bursting to single spiking sustains activation of the target once it has been "woken up" by bursting. Conversely, transitions from a powerful burst output to less powerful single-spike mode may serve to initially activate target structures but then allow other inputs to govern the target output. Given the importance of the hippocampus in processing memory and emotion, and its involvement in schizophrenia, epilepsy, and other disorders, these new insights into the regulation of its output may lead to a better understanding of numerous fundamental higher brain processes.

DOI: 10.1371/journal.pbio.0030210.g001

An acrylic painting by Don Cooper and Leah Leverich shows the transition zone between the densely packed pyramidal neurons in the CA1 region (right) and the spread-out pyramidal neurons within the subiculum (left)

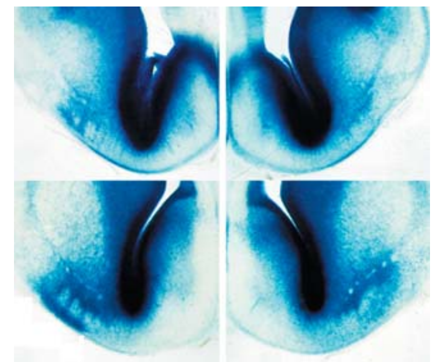
Cooper DC, Chung S, Spruston N (2005) Output-mode transitions are controlled by prolonged inactivation of sodium channels in pyramidal neurons of subiculum. DOI: 10.1371/journal.pbio.0030175

Migration and Fate Specification in the Ventral Striatum

DOI: 10.1371/journal.pbio.0030226

Like other complex organs, the brain develops in stages, under tight control of multiple genetic signals. The key to understanding brain development, therefore, lies in understanding what these signals are, where they act, and what their effects are. The SOX family of transcription factors has emerged as a major group of developmental regulators in the brain, but their exact roles are not well known. In this issue, Vasso Episkopou and colleagues show that the mouse gene *Sox1* is required for the differentiation and migration of neurons within a portion of the developing brain.

A major event in brain development is the emergence of the lateral ganglionic eminence (LGE), a bulbous protrusion whose neurons go on to form several vital brain structures, including the ventral striatum (VS), which is ultimately responsible for control of various aspects of motor, cognitive, and emotional functions. Several transcription factors have been shown to operate within the LGE precursors of the VS, but these are not present after these neurons have stopped dividing. To determine whether the protein SOX1 might be playing a role in these cells, the authors examined mice missing the SOX1 protein only in these cells. They found that the absence of the protein from these post-mitotic cells (cells after cell division) prevented normal



DOI: 10.1371/journal.pbio.0030226.g001

***Sox1* gene expression in wild-type (left) and *Sox1* mutant (right) mouse forebrain**

development of two VS structures, the striatal bridges and the olfactory tubercles.

It appeared that the defect in mice missing SOX1 from all cells was not in the proliferation of neuronal precursors, which appeared normal, but instead in their differentiation and migration. And while the previously identified factors played no role after the neurons had stopped dividing, continued expression of *Sox1* in post-mitotic neurons was required for correct specification of cell identity. Overexpression of *Sox1*, on the other hand, did not increase the normal number of olfactory tubercle–specified neurons, or alter their migration, suggesting that regulation of these cells is under more complex layers of control.

These results, combined with previous work by the same authors showing a role for *Sox1* in terminal differentiation of the mouse lens, suggest that *Sox1* and other closely related family members may help specify and maintain final post-mitotic cell identity in a variety of tissue types.

Ekonomou A, Kazanis I, Malas S, Wood H, Alifragis P, et al. (2005) Neuronal migration and ventral subtype identity in the telencephalon depend on SOX1. DOI: 10.1371/journal.pbio.0030186

A Place in the Brain for Remembering and Forgetting

DOI: 10.1371/journal.pbio.0030216

After the wounded soldier in Dalton Trumbo's *Johnny Got His Gun* slowly realizes he has no limbs, no face, ears, eyes, or mouth—but isn't dead because his mind continues to think—he desperately tries to find a way to reclaim his humanity. He decides to track time: he counts to sixty, files that minute "in one side of his mind ... and [begins] counting from one to sixty again." When he starts to wonder whether he's counting at the right speed, his mind slips "off track" and his figures disappear.

People have been known to exhibit enhanced memory in extraordinary circumstances, but human memory has its limits. Short-term retention of information, called working memory, involves doing at least two things simultaneously: paying attention to the task at hand—in the soldier's case, counting—while processing that information and deciding how to handle it—adding the numbers and keeping a running total. The secondary processing of stored information is referred to as executive control. Many studies suggest that the neural seat of both working memory and executive control—which together encompass planning, creativity, reasoning, abstraction, and most of the other higher-order cognitive properties humans like to claim as their own—lies within the prefrontal cortex. Teasing out the neural components of these overlapping processes has proven challenging.

In a new study, Jonas Rose and Michael Colombo investigate the neural basis of executive control by training homing pigeons to remember or forget a visual stimulus. Recording from the nidopallium caudolaterale (NCL), a region of the avian brain considered analogous to the mammalian prefrontal cortex, the authors show that neurons in the NCL selectively fire when the birds are told to remember and stop firing when they are told to forget.

To test the hypothesis that the NCL plays a role in executive control, the authors trained five pigeons on a directed forgetting test, a variation on the classic match-to-sample test. After viewing sample stimuli consisting of one of two shapes (a circle or dot) or colors (red or white), the birds were cued to remember or forget the sample (signaled by either a high- or low-frequency tone or one of two distinct patterns). A delay period followed these cues. If a forget cue was presented, the trial ended after the delay, and no memory test was given. If the remember cue was presented, the birds were given a memory test in which they saw two stimuli after the delay; if they responded to the sample stimulus (by pecking on a key), they were rewarded with wheat. The pigeons' NCL activity was recorded during the trials.

Eighty-three of the 124 recorded neurons were classified as delay neurons because they showed significantly different activity during the delay period, when memory was required, than during the intervals between trials, when it was not. During the remember trials, neurons showed sustained activation throughout the cue and delay periods; during the forget trials, sustained activation disappeared. To make sure the forget cue was indeed directing the birds to forget the sample stimulus, the authors ran the forget trials again, but this time, tricked the birds and gave them a memory test. The birds consistently performed worse on the forget trials than on the remember trials, confirming the forget cue's effect.

These results suggest that sustained NCL neuronal activation reflects working memory or at least some type of cognitive activity associated with a working memory task. Either way, these findings support the notion that NCL neurons play a role in executive control—what to remember and what to forget—by linking the presence or absence of neuronal activity with remembering and forgetting. And though the avian NCL and mammalian prefrontal cortex clearly differ after 320 million years of divergent evolution, Rose and Colombo make a strong case that they are similar enough to support the NCL's likely contribution to executive control in mammals as well. And they suggest that seeing such similarities between the bird and human brain forces us to reexamine not only our notions of how these structures operate but also our hubris in thinking our biology and nature is unique.

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Neurons in the avian version of the prefrontal cortex fire when pigeons perform memory tasks and may play a role in higher cognitive processes such as decision making and reasoning

Jonas Rose, Michael Colombo (2005) Rose J, Colombo M (2005) Neural correlates of executive control in the avian brain. DOI: 10.1371/journal.pbio.0030190

Microarrays Highlight Tumor–Connective Tissue Interactions in Cancer Outcomes

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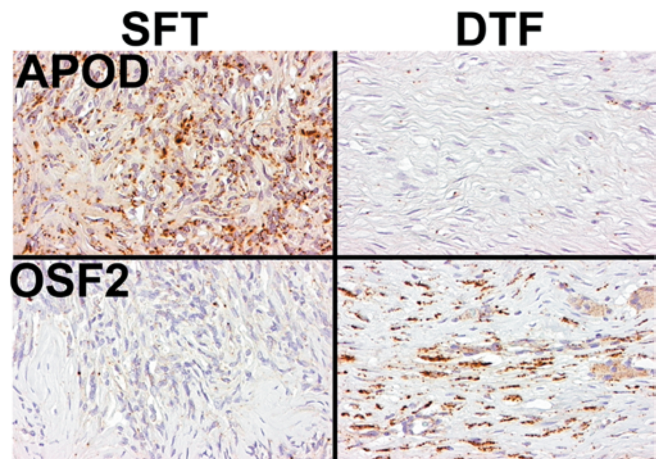
Of the 200 or so diseases collectively known as cancer, tumors of the soft tissue include some of the rarest and most diverse. Malignant soft tissue tumors, or sarcomas, and benign soft tissue tumors—which together comprise over 100 subtypes—take root in immature nerve and mesenchymal cells, which give rise to muscle, fat, cartilage, and other connective tissues. Reflecting the ubiquitous nature of connective tissue, soft tissue sarcomas can occur nearly anywhere in the body. Pathologists classify soft tumor sarcomas based on the proteins they express and on their resemblance to normal connective tissue cells (such as adipocytes, smooth muscle cells, and fibroblasts): tumors consisting of cells with cytologic features of fat cells, for example, are called liposarcomas while those forming the spindle-shaped, organized sheets typical of smooth muscle cells are called leiomyosarcomas.

But many connective cells appear too similar and express too many of the same proteins for traditional screens to distinguish among them. Assays are further complicated by the admixture of non-malignant cells, including inflammatory cells and those recruited to form new blood vessels, into the soft tissue tumor landscape.

In a new study, Robert West, Matt van de Rijn, and their colleagues investigate the notion that different types of fibroblastic tumors mirror the features of normal fibroblasts and search for tissue markers that might distinguish them. Using DNA microarrays, the authors profiled gene expression patterns in two types of fibroblastic tumors and found significant differences in the expression of functionally related genes, confirming that each tumor carries a unique genetic signature. These gene sets also appear in the matrix of normal connective tissue, or stroma, leading to the identification of two noncancerous fibroblast subtypes.

The tumors analyzed in this study—solitary fibrous tumor (SFT) and desmoid-type fibromatosis (DTF)—behave differently but consist of cells that look similar under the microscope, making them well suited to the task of identifying novel connective tissue markers. Typically benign, SFT tumors respond well to surgical excision and are thought to arise either from fibroblasts, most frequently in the thoracic cavity. DTFs, aggressive tumors found deep within the soft tissue of the trunk, abdomen, or extremities, are difficult to excise completely and are also thought to arise from fibroblasts.

Gene arrays taken from ten DTF tumors and 13 SFT tumors showed that each tumor type had distinct gene expression patterns reflecting different gene functions. For example, DTF gene profiles included many genes involved in fibrosis (scarring) and extracellular matrix remodeling, a prerequisite for the invasive behavior of aggressive DTF tumors. SFTs, on the other hand, express many genes involved in synthesis and maintenance of the basement membrane that surrounds muscle cells, blood vessels, and other specialized cells. Based on these variable expression patterns, the authors hypothesized that the tumors' cells of origin might perform different functions in normal tissue.



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Tissue samples at 600× magnification show that these two soft tissue tumors express different protein markers: solitary fibrous tumors express the APOD protein marker (top), and desmoid-type fibromatosis tumors express OSF2

Next, West et al. examined gene expression patterns in fibroblasts found in normal tissue samples to look for tumor-specific markers. As predicted, DTF markers were found in one set of tissues—related to scarring and inflammation—and SFT markers were found in another—breast and skin fibroblasts, and benign breast growths. The authors' use of soft tissue tumors to define different subsets of stromal cells is similar to past studies that used lymphomas to discover novel subsets of normal lymphoid cells.

Because normal fibroblasts can contribute to cancer progression—by providing the cellular matrix, or stroma, that supports tumor growth—the authors looked for DTF- or SFT-specific markers in the stroma of breast cancer patients. Two groups, the authors note, showed differences in the expression of DTF and SFT genes. Patients with tumors showing high DTF gene expression had the best prognosis, suggesting that tumor–stromal interactions might affect disease progression.

The difficulty of classifying fibroblasts has complicated efforts to understand how fibroblasts aid tumor progression. This study suggests that breast cancers with different stromal signatures may have different clinical outcomes, which raises many questions for future study. If the same tumor type grows on a different stromal background, will it progress differently? If so, how do tumor–stromal interactions influence progression? Thanks to the fibroblast markers identified here, scientists have new tools for exploring these questions.

West RB, Nuyten SA, Subramanian S, Nielsen TO, Corless CL, et al. (2005) Determination of stromal signatures in breast carcinoma. DOI: 10.1371/journal.pbio.0030187