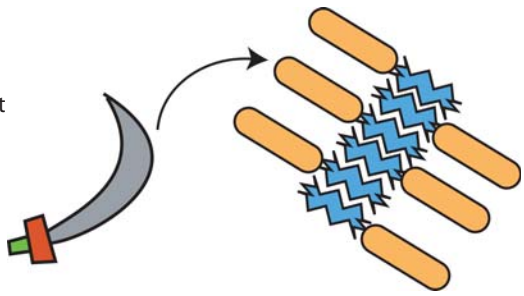
Research Digest

Synopses of Research Articles

Artificial Prions Created from Portable Control Elements

For decades, scientists accepted that the nucleic acids, DNA and RNA, packed with thousands of protein-coding genes, were the sole purveyors of genetic information; all inherited traits, from eye color to shoe size, must be stored and expressed through nucleic acid mechanisms. But prions are an exception. These misshapen proteins are capable of growing, replicating, and infecting other cells—that is, they are heritable. And all without a scrap of DNA. Most famous as the culprit behind bovine spongiform encephalopathy, or mad cow disease, prions also occur naturally in some organisms and may play important roles in their growth and development.

Prion-forming proteins normally exist as benign cellular components, such as enzymes or receptors. But they possess the innate ability to alter their three-dimensional structure, or fold, which changes their function and makes them almost impossible to destroy. Like other misfolded proteins, such as those responsible for Alzheimer's and Huntington's diseases, prions pack together and form aggregates. But what distinguishes prions from simple protein aggregates is their exponential growth and amplification, which allows them to infect new host cells. Prions grow by inducing normal proteins to alter their shape and adhere to an initial aggregate "seed." These growing masses are then thought to divide with the help of "chaperones," cellular proteins that aid in protein folding and transport, resulting in smaller prion particles called propagons. The propagons are then distributed to both mother and daughter cells during division, thereby infecting the next generation of cells. Though this theory of the prion life cycle was proposed a few years ago, scientists are still working out the underlying molecular mechanisms



Chaperone-dependent prion severing

As they report in this issue, Lev Osherovich and colleagues dissected yeast prions and found that growth and heritability are controlled by two independent and "portable" sequences. Furthermore, the heritability element seems to be the only thing that keeps slow growing protein aggregates from becoming infectious prions. Previous research showed that one end of the yeast protein, Sup35p, is critical for turning this normal housekeeping enzyme into a prion. The "prion-forming domain" of Sup35p consists of two segments: one stretch rich in the amino acids glutamine and asparagine and another made up of several, small series of amino acids, called oligopeptides. Osherovich and colleagues had earlier found another yeast protein, New1p, which had similar segments, though in reverse order.

To study the function of these sequences, the team constructed several strains of yeast, each with a small part of the prion-forming domain missing. By watching the behavior of these modified proteins, each fused to a green fluorescent protein for easy observation, the authors could infer the roles of the deleted segment.

For both Sup35p and New1p, the authors found that the area rich in glutamine and asparagine was responsible for the aggregation and growth of prions—acting like a patch of Velcro that locks the misshapen proteins together. While this had been suggested by previous research, the authors also found that this sticky

sequence only adheres to proteins that mirror its own pattern of amino acids, thereby explaining why prions from different species don't often interact, a phenomenon called the species barrier. The stretch of oligopeptide repeats in Sup35p and New1p, however, was required for the inheritance of prions—the proper division of prion masses and subsequent distribution of propagons during cell division. The authors suggest that oligopeptide repeats function as a secure binding location for the chaperone proteins, which are necessary for heritability, and thus infectiousness, of prions. Their results also help to explain why stable inheritance of prions is rare; while many proteins have stretches of amino acids similar to the described aggregation sequence, few also contain sequences like oligopeptide repeats that permit inheritance.

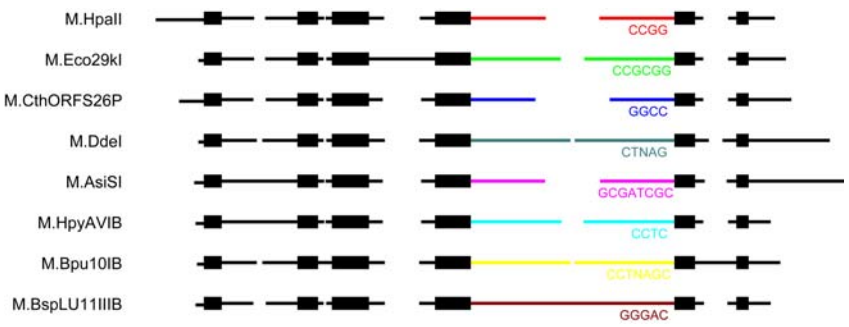
Though both the aggregation sequence and the oligopeptide repeats are required for prion growth and infection, the segments seemed to function completely separately, allowing the authors to create a synthetic prion-forming domain by combining the aggregation element of New1p with the Sup35p replication/heritability element. This artificial prion acted like New1p, again showing that it is the sticky, aggregation element that specifies which proteins will be added to the growing prion mass. Osherovich and colleagues then went on to create another artificial prion by fusing the oligopeptide repeats to an expanded polyglutamine tract, the type of aggregation sequence responsible for the toxic buildup of brain proteins in Huntington's disease. With this simple addition, the slow growing aggregate was transformed into a heritable, infectious prion.

By creating artificial hybrid prions, Osherovich and colleagues showed that the two discrete elements of prion-forming domains are portable and work together regardless of their origins. The authors suggest that other artificial prions could be used as a model system to study different types of aggregation sequences, such as those found in the human prion protein responsible for Creutzfeldt-Jakob's disease or the misshapen plaques of proteins that contribute to Alzheimer's disease.

Osherovich LZ, Cox BS, Tuite MF, Weissman JS (2004) Dissection and design of yeast prions. DOI: 10.1371/journal.pbio.0020086

"Mosaic" Genes Highlight Forces of Genome Diversity and Adaptation

Microbes are arguably the most adaptable organisms on Earth, inhabiting nearly every crevice of nearly every corner of the globe. Some invade the cavities of a wide variety of insects and other invertebrates while others colonize the skin, blood, eyes, and internal organs of animals. Still others thrive in such inhospitable places as the hydrothermal vents of the ocean floor and the Dry Valleys of Antarctica. These "simple" single-celled organisms have evolved unique molecules and strategies over some 3.5 billion years that suit life on the edge. With the sequenced genomes of nearly 140 microbial species in hand, scientists are gaining valuable insights into the nature of this adaptive diversity.



Segmentally variable genes

Adapting to such radically different niches, it appears, has produced genes with diverse functions that evolve at very different rates. Genes that code for molecules essential for fundamental cellular functions like maintaining cell metabolism and structure tend to evolve rather slowly, while genes that make proteins charged with mediating cellular responses to internal or external changes often evolve relatively quickly. Pathogenic microbes in particular rely on a flexible genome to keep a step ahead of their hosts' similarly evolving defenses in the never-ending struggle to gain adaptive advantage. This adaptability underlies the increasing antibiotic resistance of diseases like tuberculosis, as selective pressures favor the expansion of resistant bacterial populations.

Combating such problems requires a molecular understanding of bacterial infections, yet function has been ascribed to only a fraction of the genes found in microbial genomes. One approach to improve functional analyses of genome sequences combines bioinformatics with experimental methods. With such collaborations in mind, Yu Zheng, Richard Roberts, and Simon Kasif have developed a computational approach to help filter out the genetic noise and home in on genomic regions likely to contain clues to gene function. Their method relies on a novel way of classifying genes that flags sequences likely to reward biochemical and genetic efforts to analyze gene function.

Many comparative genomic studies have focused on looking for sequence "motifs" that correlate with well-characterized protein sequences (that is, the amino acid sequence) and predicting function based on their similarity to the known protein sequences. Zheng, Roberts, and Kasif took a different approach, classifying genes based on their sequence variation. The researchers analyzed 43 fully sequenced microbial genomes and, after determining the degree of conservation or divergence among similar genes in different species, divided the genes into three broad categories: rapidly evolving genes unique to a particular species; highly conserved genes; and "segmentally variable," or mosaic, genes. Stipulating that the boundaries between the categories are somewhat blurred, Zheng et al. define segmentally variable genes as regions that show a mosaic pattern of one or more rapidly evolving, variable regions interspersed with conserved regions. Based on evidence suggesting that retained variable regions tend to serve a

function, the researchers predicted that these mosaic genes, with their highly variable, fast-evolving regions, would shed light on the forces that shape genome diversity and adaptation.

For most of the microbes analyzed, mosaic genes accounted for about 8–20% of their genomes. Selecting several large families of mosaic genes, Zheng et al. explored the relationship between genes with known function and the structure of their variable regions. Noting an overabundance of particular functional categories in different species—such as signaling proteins that come into either direct or indirect contact with the cell's environment—the researchers speculate that the variable regions may constitute an adaptive layer for the microbe, as they not only "play a key role in mediating interactions with other molecules" but also support a microbe's ability to adapt to its particular niche. Several bacteria species, for example, contain roughly 40% more mosaic sensor genes involved in cell motility, which the authors attribute to the microbes' "expanded ability to detect different chemical signals and find favorable environments."

This regional variability appears to reflect the influence of selective pressures that fuel diversity through ongoing interactions with other rapidly evolving molecules in the environment, adding another source of genetic adaptability as cells adjust to new environments and outmaneuver pathogenic threats. While many of the mosaic genes identified encode proteins involved in host-pathogen interactions, defense mechanisms, and intracellular responses to external changes, their function is only broadly understood. While Zheng et al. cannot say to what extent variability affects function—Is extreme variability required for diversity or can modest variation suffice?—they are refining their classification of segmentally variable genes to address such questions. Until then, the authors' "mosaic" approach to understanding gene function promises to improve efforts to annotate the volumes of sequenced genomes on hand, offering biologists a much-needed tool to sift through the mountains of genomic datasets and identify promising targets for further study.

Zheng Y, Roberts RJ, Kasif S (2004) Segmentally variable genes: A new perspective on adaptation. DOI: 10.1371/journal.pbio.0020081

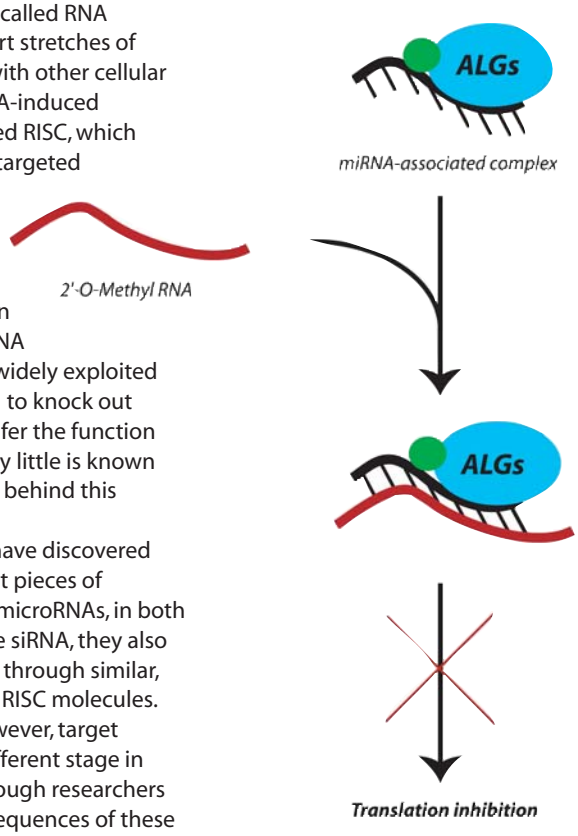
Exploring Small RNA Function

Regulation of gene expression—deciding how much of what proteins are produced in the cell—is controlled by a myriad of different molecules. One type of naturally occurring regulatory molecule is small interfering RNA (siRNA), which selectively disrupts the production of a protein it is programmed to recognize, a process called RNA interference. These short stretches of nucleotides combine with other cellular proteins to form an RNA-induced silencing complex, called RISC, which locates and destroys a targeted messenger RNA—the molecule that carries a protein recipe from the nucleus to the site of production in the cytoplasm. While RNA interference has been widely exploited by researchers as a tool to knock out gene expression and infer the function of missing proteins, very little is known about the mechanisms behind this regulatory process.

Recently, biologists have discovered hundreds of other short pieces of regulatory RNA, called microRNAs, in both plants and animals. Like siRNA, they also affect gene expression, through similar, possibly even identical RISC molecules. Animal microRNAs, however, target messenger RNA at a different stage in protein production. Though researchers have determined the sequences of these microRNAs, uncovering their function—that is, which protein they interrupt and, in turn, what the interrupted protein does—has progressed slowly and sporadically without any decisive tool to study them. Only four animal microRNAs have known biological functions, despite the intense level of work going on in this field.

In this issue, György Hutvágner and colleagues report a rapid and reliable method for knocking out both siRNAs and microRNAs and thereby exploring their functions. The authors found that a short stretch of nucleotides, called a 2′-*O*-methyl oligonucleotide, whose sequence mirrors the targeted siRNA or microRNA, could bind and inhibit their function, allowing researchers an unprecedented glimpse at the regulatory roles and mechanisms behind RNA interference.

The authors first tested their oligonucleotide design against an siRNA known to interfere with production of the firefly protein, luciferase—this luminescent protein is often used as a "reporter," lighting up when cells successfully produce the protein. Any interference means the glow is gone. Using extracts from fruitfly embryos as the test-bed, the researchers mixed in the luciferase-associated siRNA and the



Inhibiting miRNA

sequence-specific oligonucleotide. What holds these two molecules together is complementary base-pairing, the same force that holds two molecules of DNA together. As predicted, the oligonucleotide inhibited RISC activity—it could no longer silence the production of luciferase.

Because the authors could easily control the concentration of both the siRNA and the oligonucleotide inhibitor in these fly extract experiments, they were able to answer several questions about how these two molecules interact. They found that adding greater and greater concentrations of siRNA molecules did not result in equally great numbers of RISC; the process became saturated, indicating that a protein in the RISC assembly pathway limits

production. Furthermore, the authors saw a marked 1:1 relationship between the concentration of the oligonucleotide and the concentration of RISC, indicating that each inhibitor binds to one RISC molecule in order to inactivate, a binding that appears to be irreversible. The results also showed that, though RISC molecules bind to the inhibitor through complementary base-pairing, a very different and more complex interaction is used by RISC molecules to find and bind their natural interference targets.

The authors then went on to use the luciferase siRNA to test the function of their oligonucleotide inhibitor in cultured human cells, which had been engineered to contain the luciferase gene. This in vivo experiment, using living and metabolizing cells, showed results similar to those with fruitfly extracts. But the real test for these inhibitors was to use them in a whole animal against a previously identified microRNA where the outcome of its inactivation was already known.

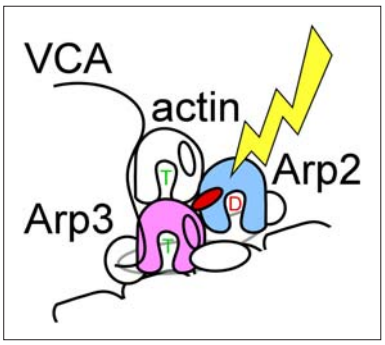
Hutvágner and colleagues constructed an oligonucleotide inhibitor based on the sequence of a microRNA called *let-7*, which blocks the production of the protein Lin-41 and is important for proper developmental timing in roundworm larvae. Larvae injected with the oligonucleotide had the exact features of a *let-7* deficient worm, showing that the inhibitor did indeed block this microRNA's function. The authors also used the oligonucleotides to provide evidence that two proteins, previously suggested to be involved with *let-7*, were directly associated with its interfering activity.

Using the technique described here, scientists could make rapid headway toward uncovering the biological functions of hundreds of microRNAs, their accessory RISC proteins, and even the proteins and genes they are programmed to interrupt. Furthermore, finding that RISC production is saturable could have significant implications for genetic studies that use RNA interference to uncover the function of sequenced, but unknown, genes; knowing the minimum required concentration of siRNA, researchers can avoid a buildup and any unwanted cell activity that goes along with it.

Hutvágner G, Simard MJ, Mello CC, Zamore PD (2004) Sequence-specific inhibition of small RNA function. DOI: 10.1371/journal.pbio.0020098

A Mechanism for Adding the First Link in a Nascent Actin Filament Chain

The capacity for self-generated movement is a defining characteristic of animal life. With the molecular components of cellular locomotion conserved in organisms from protozoa to vertebrates, directed cell motility appears to be an ancient cell process, likely dating back a billion years. Most directed motion relies on the assembly, or polymerization, of actin proteins into filaments. Actin is one of the most abundant proteins in cells; about half of the cellular concentration of actin is bound together in filaments at any given time while the other half floats freely as "monomers" in the cytoplasm. The erection and demolition of actin filaments directs the cell motility that lays down the remarkable million miles of nerve cells that form the nervous system and drives a variety of fundamental biological processes, from effective immune response to embryonic development. Mutations in proteins that regulate actin assembly can lead to the abnormal cell migration associated with metastatic cancer. The actin cytoskeleton also provides the structural support for animal cells that the cell wall provides for plants.



Actin addition

The molecular mechanisms underlying actin assembly and cell motility remained obscure until 1994, when Thomas Pollard and his colleagues discovered the protein complex that initiates actin polymerization. Called actin-related protein 2/3 (Arp2/3) complex, this molecular machine consists of seven subunits, including the two actin-related proteins. Free actin monomers are primed for rapid polymerization, but polymerization must be initiated by the Arp2/3 complex in a process referred to as nucleation. To nucleate a new filament, the Arp2/3 complex must be activated, a job accomplished by a family of proteins called WASP (after Wiskott Aldrich Syndrome, a genetic disease characterized by defects in platelet development and lymphocyte function). WASP proteins bind to both the Arp2/3 complex and an actin monomer. The Arp2/3 complex also binds two molecules of adenosine triphosphate (ATP) on the Arp2 and Arp3 subunits. ATP releases energy in a process called hydrolysis, which drives most energy-dependent processes, from actin polymerization to muscle contraction. The precise mechanisms governing Arp2/3 activation and nucleation are not known. Now Mark Dayel and Dyche Mullins show where hydrolysis occurs during this crucial first step in polymerization and use this finding to investigate the mechanisms that drive nucleation.

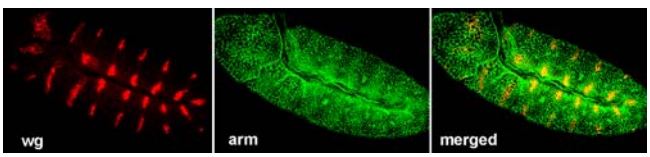
In previous experiments, Dayel and Mullins found that Arp2/3 appears to require hydrolysable ATP to effect nucleation. To determine when and if ATP hydrolysis occurs on the Arp2/3 complex, Dayel and Mullins developed a technique that allowed them to analyze the Arp2 and Arp3 subunits separately. Dayel and Mullins discovered that hydrolysis occurs only on the Arp2 subunit of the complex and that it happens during the step when WASP initiates the nucleation of a new filament. The researchers then used ATP hydrolysis on Arp2 to dissect the mechanism by which WASP activates the Arp2/3 complex and develop a model of nucleation. (All previous techniques required actin polymerization to monitor the activity of the Arp2/3 complex, but this technique offers a way to decouple activation from polymerization.) They find that WASP proteins activate the Arp2/3 complex by coordinating its interaction with an actin monomer—the first monomer of the new filament.

By developing a novel technique to monitor activation of the Arp2/3 complex, the authors contribute a new tool for further investigations of this central part of the cellular motility machinery. And by showing how Arp2/3 is activated, they offer important insights into the workings of a multiprotein cellular machine and the mechanisms that cells enlist to control their shape and motility---which could suggest potential drug targets to inhibit the abnormal cell movement characteristic of cancer and other diseases.

Dayel MJ, Mullins RD (2004) Activation of Arp2/3 complex: Addition of the first subunit of the new filament by a WASP protein triggers rapid ATP hydrolysis on Arp2. DOI: 10.1371/journal.pbio.0020091

Wnt Signaling Relies on Nuclear Armadillo

A couple of years ago, a paper was published in a high-profile journal that challenged a long-established model of cell signaling. While researchers in the field mostly greeted the results with skepticism, some went into the lab to investigate the discrepancy. Many elements of this pathway, called the Wnt pathway, have been well characterized. The standard model of Wnt signaling holds that when the Wnt protein binds to its receptor, it initiates a labyrinthine signaling cascade that sends a protein called β-catenin into the cell's nucleus where, together with a protein complex, it initiates transcription. In the absence of this signal, β-catenin binds to an inactivating complex in the cytoplasm and is targeted for degradation. The paper that disputed this view suggested that β-catenin can effect gene expression without entering the nucleus and that it can activate the Wnt pathway while tethered to the cell membrane.



Striped expression of wingless and armadillo

Before that paper was published, Nicholas Tolwinski and Eric Wieschaus had shown that β-catenin, also known as Armadillo (Arm) in the fruitfly, is sent into the nucleus in response to Wnt signaling. Upon entering the nucleus, Arm interacts with a second protein complex to activate transcription. Now Tolwinski and Wieschaus have reexamined the function of Arm in the fruitfly and have demonstrated that the pathway "in fact does depend on the nuclear localization of β-catenin." While their paper was in the final stages of acceptance, the dissenting paper was retracted, after it was learned that the results had been fabricated. Tolwinski and Wieschaus' findings confirm what had already been known about Arm's role in Wnt signaling and also fill in important details about how it works.

Multicellular organisms rely on elaborate communication networks of signaling proteins and enzymes to exchange information between cells. The Wnt signaling pathway regulates the expression of a host of different genes during embryogenesis to control body patterning and cell differentiation in organisms from fruitflies to mammals. Miscommunications in this tightly regulated pathway contribute to a variety of developmental defects and cancers.

In the developing fruitfly, Wnt signaling is normally restricted to the front of each larval segment, where it produces a smooth surface; the rear of the segments, where Wnt signaling is absent, is hairy. If Wnt signaling is overexpressed, it produces fruitfly larvae with only smooth segments; lack of Wnt signaling produces hairy segments. Using the smooth phenotype as a measure of Wnt signaling, Tolwinski and Wieschaus delved deeper into the role of Arm in this signaling process.

These experiments are complicated because Arm functions not just in Wnt signaling, but also in cell adhesion. The trick is to make the endogenous Arm (the version encoded by the fly genome) defective for signaling, while leaving the cell adhesion functions fairly normal. Set against this "background," an additional Arm protein is expressed that is tethered to the membrane; it still retains the protein domains required for signaling, but it's stuck on the inside of the membrane and can't move into the nucleus. In this background, any signaling in response to Wnt must mean that Arm can signal to the nucleus without actually having to get inside. Tolwinski and Wieschaus prove—again—that this is not the case. They do this by showing that the weak, medium, and strong endogenous Arm mutants—these classifications reflect the severity of the mutations' effects—have different effects on signaling in the presence of the membrane-tethered Arm.

It's clear, they argue, that tethered Arm cannot signal on its own and must somehow be helping the weaker mutants signal. To further investigate how the tethered Arm activates the endogenous mutants, Tolwinski and Wieschaus developed two new and "cleaner" Arm mutants that impair Arm's signaling ability but have no effect on its cell adhesion function. The tethered Arm could not produce a completely smooth phenotype with these nonsignaling endogenous mutants. These experiments, the authors conclude, indicate that the tethered form of Arm produces its transcriptional effects by activating the endogenous Arm protein. Normal activation of the pathway liberates Arm proteins from the inactivating complex, which allows them to enter the nucleus and activate transcription. Tethered Arm appears to accomplish this by sequestering the inactivating complex at the cell membrane, preventing it from interfering with endogenous Arm.

Even though Tolwinski and Wieschaus started these experiments based on what turned out to be fabricated results, their investigations produced valuable contributions. They not only reaffirm the standard model of Wnt signaling, but reveal important new insights into the workings of a major player in the pathway.

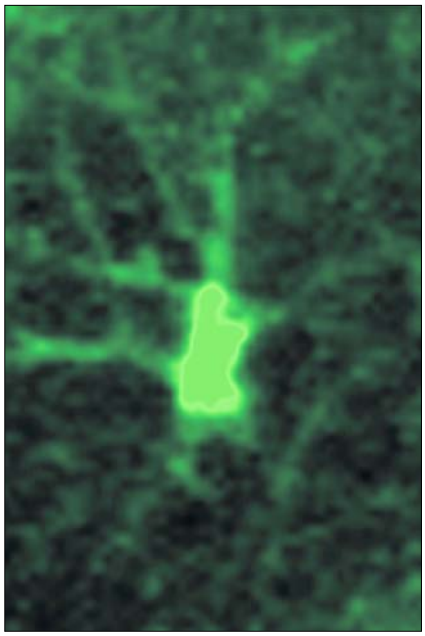
Tolwinski NS, Wieschaus E (2004) A nuclear function for Armadillo/β-catenin. DOI: 10.1371/journal.pbio.0020095

A Window into the Brain Demonstrates the Importance of Astrocytes

Did you ever wish you could peek inside someone's brain and see what was going on in there? In research reported in this issue of *PLoS Biology*, Hajime Hirase and his colleagues at Rutgers University have done just that by focusing their microscope on the brains of living rats in order to examine how certain cells called astrocytes function in vivo.

In the longstanding quest to understand how the brain works, scientists have focused on neurons. Neurons conduct action potentials, electrical signals that transmit information in the nervous system. But the brain also contains several other types of cells called glia. (*Glia* is derived from the Latin for "glue"; these cells were thought to "hold it all together.") One type of glial cell, the astrocyte (named for its starlike shape), is the most populous cell in the brain and forms an intimate association with neurons and their synapses. It was thought that these cells played a supporting role in the brain, ensuring the proper chemical environment for synapses.

Recent research, however, has suggested that astrocytes and other glial cells may play a more significant role. When examining astrocytes cultured in the lab, scientists have observed behavior suggesting that astrocytes can communicate with neurons. Though astrocytes cannot propagate electrical signals like neurons do, they can sense the transmission of such signals at the synapse between two neurons. Furthermore, astrocytes are able to propagate a different kind of signal, a chemical signal based on the release of calcium ions. Calcium signaling is a mechanism of chemical signaling that has been observed in many other cell types. The exact properties of neuron–astrocyte communication, however, are not clear because different preparations of these tissues have yielded different results. It has also not been established that this type of communication occurs in the living brain.



Astrocyte in the cerebral cortex

To explore such questions, Hirase and colleagues have taken the next step by investigating the calcium signaling properties of astrocytes in the brains of living rats. To accomplish this feat, the researchers used a combination of two technologies. They monitored calcium signaling using a fluorescent dye called Fluo-4, which fluoresces in response to calcium ions. Then they used a special type of microscope called a two-photon laser scanning microscope to visualize the dye. Since this type of microscope uses a lower energy laser, it can image the dye in living tissue without causing harm.

The researchers applied the dye to the brains of anesthetized rats, washed out the excess dye that had not penetrated into cells, and then imaged the tissue under the microscope. They first confirmed that they indeed were examining astrocytes and noticed that cells displayed a moderate level of baseline calcium signaling activity. They then used a drug called bicuculline to stimulate neurons and observed a significant increase in the calcium signaling activity of the astrocytes. Because bicuculline only affects neurons, this implies that the astrocytes are responding to the activity of the neurons. The researchers also found that neighboring astrocytes often also displayed coordinated calcium signaling activity, suggesting that the communication among astrocytes is facilitated by increased neuronal activity.

This research confirms the complexity of astrocyte signaling functions in the living brain and demonstrates that astrocytes play far more than a supporting role in brain function. It also establishes an important experimental system for scientists seeking to understand how these distinct elements of the brain—neurons and astrocytes—work together. Though this research makes it clear that signaling exists both among astrocytes and between neurons and astrocytes, scientists have yet to understand the effect of this signaling. Some possibilities include regulation of synapse formation, modification of synaptic strength, or more complicated roles in information processing resulting from the coordination of neuronal activity. Future research using this and other systems will help reveal these functions.

Hirase H, Qian L, Barthó P, Buzsáki G (2004) Calcium dynamics of cortical astrocytic network in vivo. DOI: 10.1371/journal.pbio.0020096

Neural Basis of Solving Problems with Insight

If you're one of those insufferable people who can finish the Saturday *New York Times* crossword puzzle, you probably have a gift for insight. The puzzles always have an underlying hint to solving them, but on Saturdays that clue is insanely obtuse. If you had all day, you could try a zillion different combinations and eventually figure it out. But with insight, you'd experience the usual clueless confusion, until—voilà—the fog clears and you get the clue, which suddenly seems obvious. The sudden flash of insight that precedes such "Aha!" moments is characteristic of many types of cognitive processes besides problem-solving, including memory retrieval, language comprehension, and various forms of creativity. Although different problem-solving strategies share many common attributes, insight-derived solutions appear to be unique in several ways. In this issue, researchers from Northwestern and Drexel Universities report on studies revealing a unique neural signature of such insight solutions.

Mark Jung-Beeman, John Kounios, Ed Bowden, and their colleagues recount the storied origin of the term *Eureka!*, which Archimedes reportedly shouted upon realizing that water displacement could be used to compute density. Illustrating the strong emotional response elicited by such a sudden insight, Archimedes is said to have run home from the baths in euphoric glee—without his clothes.

Among other characteristics that typically distinguish insight from "noninsight" solutions, people feel stuck before insight strikes; they can't explain how they solved the problem and might say they were not even thinking about it; the solution appears suddenly and is immediately seen as correct. But are the neural processes involved in arriving at a solution through insight actually distinct from those related to more mundane problem-solving?

Recent findings suggest that people think about solutions, at an unconscious level, prior to solving insight problems, and that the right cerebral hemisphere (RH) appears to be preferentially involved. Jung-Beeman et al. predicted that a particular region of the RH, called the anterior superior temporal gyrus (aSTG), is likely involved in insight because it seems critical for tasks that

Insight lights up the brain

require recognizing broad associative semantic relationships—exactly the type of process that could facilitate reinterpretation of problems and lead to insight.

To test this hypothesis, Jung-Beeman et al. mapped both the location and electrical signature of neural activity using functional magnetic resonance imaging (fMRI) and the electroencephalogram (EEG). In the first experiment, thirteen people were given three words (*pine, crab, sauce*) and asked to think of one word that would form a compound word or phrase for each of the words (can you figure it out?). Neural activity was mapped with fMRI while the participants were given 124 similar word problems—which can be solved quickly with or without insight, and evoke a distinct Aha! moment about half the time they're solved. Subjects pressed a button to indicate whether they had solved the problem using insight, which they had been told leads to an Aha! experience characterized by suddenness and obviousness.

While several cortical regions showed about the same heightened activity for both insight and noninsight-derived solutions, only the aSTG in the RH showed a robust insight effect. Given that neural activity in this area also increased when subjects first encountered the problem (perhaps reflecting unconscious processing), the authors conclude that the increase does not simply reflect the emotional jolt associated with insight.

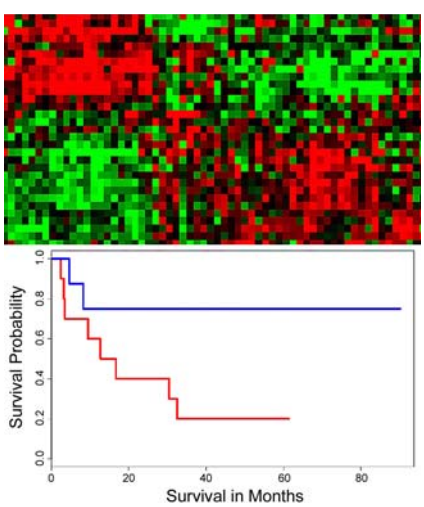
In a second experiment, 19 new participants engaged in the same type of problem-solving tasks as the first group while their brain waves were measured with an EEG. The researchers then analyzed the EEG recordings to look for differences between insight and noninsight solutions in brain wave activity. The researchers found that 0.3 seconds before the subjects indicated solutions achieved through insight, there was a burst of neural activity of one particular type: high-frequency (gamma band) activity that is often thought to reflect complex cognitive processing. This activity was also mapped to the aSTG of the RH, providing compelling convergence across experiments and methods.

Problem-solving involves a complex cortical network to encode, retrieve, and evaluate information, but these results show that solving verbal problems with insight requires *at least* one additional component. Further, the fact that the effect occurred in RH aSTG suggests what that process may be: integration of distantly related information. Distinct neural processes, the authors conclude, underlie the sudden flash of insight that allows people to "see connections that previously eluded them."

Jung-Beeman M, Bowden EM, Haberman J, Frymiare JL, Arambel-Liu S, et al. (2004) Neural activity when people solve verbal problems with insight. DOI: 10.1371/journal.pbio.0020097

Predicting Cancer Patient Survival with Gene Expression Data

Cancer specialists often talk about cancer as an umbrella term for over 200 different diseases, each having unique characteristics. But even these categories are too broad, as the same type of cancer can take very different paths in different people. It's not uncommon, for example, for a tumor to grow aggressively in one patient and stabilize or regress in another, even though their tumors are indistinguishable and are treated in the same way. Researchers have traditionally diagnosed and treated cancer based on microscopic analysis of cell size and shape, a method that's especially difficult for very closely related cancers, such as non-Hodgkin's lymphoma, which has 20 subtypes. As scientists learn more about the molecular alterations in cancer, they're beginning to establish cancer subtypes based on the underlying molecular footprint of a tumor. Four years ago, DNA microarray analysis revealed that the most common subtype of non-Hodgkin's lymphoma is in fact two separate diseases. Though the tumor cells of both cancers appear large and diffusely dispersed in a tissue sample under a microscope, each has a distinct genetic profile, possibly explaining why only 40% of patients with this subtype respond to the standard chemotherapy treatment.



Selecting expression profiles that can predict cancer outcome

Such molecular pathology has led to the discovery of subtypes of several different tumor types and has successfully identified patients with different survival times. But such correlations work best when cancer subtypes based on genetic profiles are already known. If you know that different subtypes exist and which patients belong to which subtype, then you can build a statistical model to diagnose such cancers in future patients. But in most situations, clinicians don't know either of these variables—or even whether such a subtype exists—information that is crucial to developing effective diagnostic and treatment protocols. Statistical methods to identify such subtypes exist, but they can generate classifications that lack clinical relevance. Now Eric Bair and Robert Tibshirani describe a procedure that combines both gene expression data and the patients' clinical history to identify biologically significant

cancer subtypes and show that this method is a powerful predictor of patient survival.

Their approach uses clinical data to identify a list of genes that correspond to a particular clinical factor—such as survival time, tumor stage, or metastasis—in tandem with statistical analysis to look for additional patterns in the data to identify clinically relevant subsets of genes. In many retrospective studies, patient survival time is known, even though tumor subtypes are not; Bair and Tibshirani used that survival data to guide their analysis of the microarray data. They calculated the correlation of each gene in the microarray data with patient survival to generate a list of "significant" genes and then used these genes to identify tumor subtypes. Creating a list of candidate genes based on clinical data, the authors explain, reduces the chances of including genes unrelated to survival, increasing the probability of identifying gene clusters with clinical and thus predictive significance. Such "indicator gene lists" could identify subgroups of patients with similar gene expression profiles. The lists of subgroups, based on gene expression profiles and clinical outcomes of previous patients, could be used to assign future patients to the appropriate subgroup.

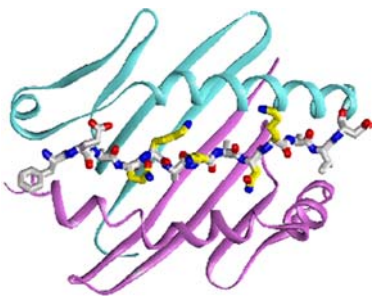
An important goal of microarray research is to identify genetic profiles that can predict the risk of tumor metastasis. Being able to distinguish the subtle differences in cancer subtype will help doctors assess a patient's risk profile and to prescribe a course of treatment tailored to that profile. A patient with a particularly aggressive tumor, for example, would be a candidate for aggressive treatment, while a patient whose cancer seems unlikely to metastasize could be spared the debilitating side effects of aggressive anticancer therapies. By providing a method to cull the thousands of genes generated by a microarray to those most likely to have clinical relevance, Bair and Tibshirani have created a powerful tool to identify new cancer subtypes, predict expected patient survival, and, in some cases, help suggest the most appropriate course of treatment.

Bair E, Tibshirani R (2004) Semi-supervised methods to predict patient survival from gene expression data. DOI: 10.1371/journal.pbio.0020108

Phage Display Libraries Identify T Cells

Doctors and researchers often look for the rapid proliferation of T cell populations, key defensive players in the immune system, as a telltale sign that the body is working hard to fend off a foreign threat. Every one of these circulating white blood cells carries a T cell receptor (TCR) that binds to a specific protein, or antigen, when displayed on the surface of a cell. A match between TCR and displayed antigen results in the cell's death and the subsequent expansion of T cell clones, all programmed to recognize the original offending protein. Some TCRs bind and expand in response to pathogenic antigens, such as viral or bacterial proteins. But T cells can also react and proliferate inappropriately in response to the body's own proteins, leading to destructive autoimmune diseases such as multiple sclerosis, which is characterized by immune system attacks on nervous tissue. Self-recognizing TCRs, however, can also target and destroy tumors—though full activation of these T cells is inconsistent and poorly understood.

Identifying the particular antigen behind an exploding population of T cells is invaluable for finding the source of autoimmune diseases and studying immune responses to cancer. But it's a laborious and time-consuming process, as researchers are faced with the prospect of sifting through millions upon millions of possible matches between TCRs and their prospective antigen epitopes—the part of the antigenic molecule to which the receptor binds. Now, as they report in this issue of *PLoS Biology*, Frances Crawford and colleagues have developed a novel method for rapidly identifying TCR mimotopes—peptide sequences similar or identical to epitopes that also elicit the immune response—which can be used to determine the antigen of a given T cell population.



Peptide display

Working backwards, the team started off with two different T cell clones that had been previously selected for with a known antigen—a peptide called p3K. One clone was derived from mice genetically engineered to have broadly reactive T cells; the other, a conventional clone, was much more sensitive to the precise molecular structure of p3K.

Crawford and colleagues then created a "peptide library" comprising more than 30,000 baculoviruses (viruses that selectively target insect cells), each one carrying a slightly different version of the p3K gene, varied in regions of the peptide known to be important for TCR binding. These p3K genes were embedded within a major histocompatibility complex (MHC) gene—a type of cell surface protein that holds displayed antigens and is also important for proper TCR recognition. The team then unleashed their virus library onto insect cells that, once infected, began to produce the specific peptide–MHC complexes encoded on the viral DNA. The insect cells then shuttled these proteins to their surfaces, resulting in a vast array of cells that each displayed a unique variant of the p3K–MHC complex. This "display library" was then incubated with fluorescently labeled TCRs from the two different clones. By observing and isolating the insect cells that lit up, the researchers could see which of the thousands of cells displaying peptide–MHC possessed a mimotope capable of binding a TCR. Because the genetic information about the displayed complex was still stored within the virus-infected cell, the researchers could determine the full peptide sequence responsible for the identified mimotopes.

Confirming the effectiveness of their method, the results of the fluorescence experiments echoed the authors' original characterizations about the two populations of T cells. The broadly reactive TCR bound to several different uniquely displayed complexes; it had 20 mimotopes. The conventional TCR, however, bound only to one peptide–MHC complex, an almost perfect match to the original p3K peptide. Though this study was based on a known antigen and epitope (which allowed verification of the method), the baculovirus display library technique described here could easily be used on T cell populations with unknown antigens. With such a tool, researchers could, for example, identify the antigens connected with tumor-fighting T cells and, through inoculation, possibly induce the production of similar T cells in cancer patients who lack them.

Crawford F, Huseby E, White J, Marrack P, Kappler JW (2004) Mimotopes for alloreactive and conventional T cells in a peptide–MHC display library. DOI: 10.1371/journal.pbio.0020090

Emergence of a Peaceful Culture in Wild Baboons

For most animal species, behavioral attributes are largely the product of interactions between genes and environment, with behavioral patterns preserved by natural selection. Birds, for example, know instinctively what type of nest to build for their offspring; salamanders don't need lessons to swim. But when it comes to primates—including humans—a good deal of behavior is learned. Primates exhibit a wide range of behaviors, not just among species but also among populations and even individuals. Yet the nature versus nurture debate still rages, particularly when it comes to understanding the roots of aggression. While bonobos are famous for using sex to resolve disputes, aggression is far more common in most primate species—again humans included. Our closest relative, the chimpanzee, has a reputation for being among the most belligerent, with rhesus monkeys and baboons not far behind. For many of these species, bouts of violence are often followed by gestures of reconciliation, such as grooming or, in the case of chimps, kissing. Since most primates live in social groups, it may be that such conciliatory measures serve to maintain some semblance of social structure, offsetting the disruptive effects of aggression. (To learn more about primate behavior and aggression, see the primer by Frans de Waal in this issue [DOI: 10.1371/journal.pbio.0020101].)

In baboons, "grooming" is a socially rewarding behavior. (Photograph, with permission, by Robert Sapolsky.)

Primatologists characterize these behavioral differences as "cultural" traits, since they arise independent of genetic or environmental factors and are not only shared by a population (though not necessarily a species) but are also passed on to succeeding generations. Such cultural traditions have been documented in African chimp populations, which display over 39 behaviors related to "technology" (such as using stones to crack nuts), grooming, and courtship. While most of these cases involve either tools, foraging, or communication, Robert Sapolsky and Lisa Share report evidence of a higher order cultural tradition in wild baboons in Kenya. Rooted in field observations of a group of olive baboons (called the Forest Troop) since 1978, Sapolsky and Share document the emergence of a unique culture affecting the "overall structure and social atmosphere" of the troop.

In his book *A Primate's Memoir*, Sapolsky studied the activities and lifestyle of the Forest Troop to explore the relationship between stress and disease. In typical baboon fashion, the males behaved badly, angling either to assume or maintain dominance with higher ranking males or engaging in bloody battles with lower ranking males, which often tried to overthrow the top baboon by striking tentative alliances with fellow underlings. Females were often harassed and attacked. Internecine feuds were routine. Through a heartbreaking twist of fate, the most aggressive males in the Forest Troop were wiped out. The males, which had taken to foraging in an open garbage pit adjacent to a tourist lodge, had contracted bovine tuberculosis, and most died between 1983 and 1986. Their deaths drastically changed the gender composition of the troop, more than doubling the ratio of females to males, and by 1986 troop behavior had changed considerably as well; males were significantly less aggressive.

After the deaths, Sapolsky stopped observing the Forest Troop until 1993. Surprisingly, even though no adult males from the 1983–1986 period remained in the Forest Troop in 1993 (males migrate after puberty), the new males exhibited the less aggressive behavior of their predecessors. Around this time, Sapolsky and Share also began observing another troop, called the Talek Troop. The Talek Troop, along with the pre-TB Forest Troop, served as controls for comparing the behavior of the post-1993 Forest Troop. The authors found that while in some respects male to male dominance behaviors and patterns of aggression were similar in both the Forest and control troops, there were differences that significantly reduced stress for low ranking males, which were far better tolerated by dominant males than were their counterparts in the control troops. The males in the Forest Troop also displayed more grooming behavior, an activity that's decidedly less stressful than fighting. Analyzing blood samples from the different troops, Sapolsky and Share found that the Forest Troop males lacked the distinctive physiological markers of stress, such as elevated levels of stress-induced hormones, seen in the control troops.

In light of these observations, the authors investigated various models that might explain how the Forest Troop preserved this (relatively) peaceful lifestyle, complete with underlying physiological changes. One model suggests that nonhuman primates acquire cultural traits through observation. Young chimps may learn how to crack nuts with stones by watching their elders, for example. In this case, the young baboon transplants might learn that it pays to be nice by watching the interactions of older males in their new troop. Or it could be that proximity to such behavior increases the likelihood that the new males will adopt the behavior. Yet another explanation could be that males in troops with such a high proportion of females become less aggressive because they don't need to fight as much for female attention and are perhaps rewarded for good behavior. But it could be that the females had a more direct impact: new male transfers in the Forest Troop were far better received by resident females than new males in the other troops.

Sapolsky and Share conclude that the method of transmission is likely either one or a combination of these models, though teasing out the mechanisms for such complex behaviors will require future study. But if aggressive behavior in baboons does have a cultural rather than a biological foundation, perhaps there's hope for us as well.

Sapolsky RM, Share LJ (2004) A pacific culture among wild baboons: Its emergence and transmission. DOI: 10.1371/journal.pbio.0020106

Evaluating Disease Trends in Marine Ecosystems

After the recent mad cow scare in the United States, 61% of Americans said they would start eating more fish, according to a *Wall Street Journal Online* poll. The respondents may not know that populations of large predatory fish, such as tuna, swordfish, and marlin, have declined 90% over the past 50 years or that less-prized species are increasingly overfished. Or that ever more fish and seafood species show rising levels of mercury contamination, rendering them unfit for human consumption—and contaminating other organisms in the ocean food chain. Humans are also affecting marine life in unexpected ways, as when large numbers of seals in Antarctica in 1955 and in Siberia in 1987 succumbed to canine distemper virus, presumably contracted from domestic dogs. In 2000, more than 10,000 Caspian seals—which also had contact with domestic dogs—died of the same virus. Such human incursions cause even more damage by exacerbating the effects of naturally occurring parasitic and pathogenic diseases that already wreak havoc as they ripple through the food chain.

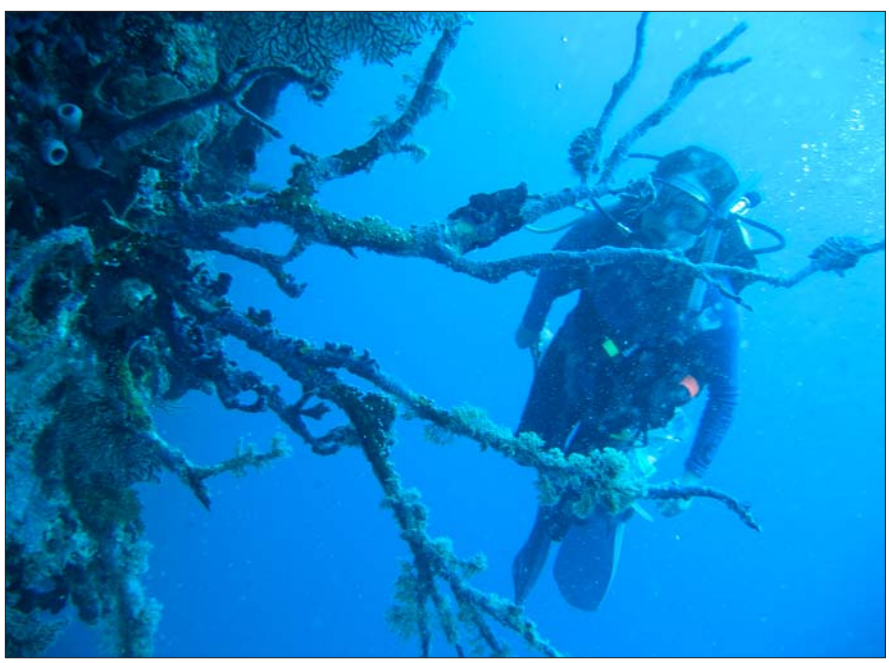
With recent studies suggesting that disease rates have increased over the past 30 years—and are expected to increase even more, thanks to global climate change—prospects for protecting marine ecosystems depend on understanding the causes and nature of these disease outbreaks. While all indicators point to a real increase in disease rates, scientists have no baseline data to measure these increases against and so cannot directly test the hypothesis that marine diseases are increasing. Now Jessica Ward and Kevin Lafferty report a method that uses the recorded incidence of disease as a proxy for baseline data to identify disease trends in major groups of marine organisms.

Ward and Lafferty conducted an online search of 5,900 journals published from 1970 to 2001 for reports of disease in nine taxonomic groups: turtles, corals, mammals, urchins, mollusks, seagrasses, decapods (crustaceans), sharks/rays, and fishes. Their approach takes into account three potentially confounding factors in determining trends in this type of search. Fluctuations in publication numbers could skew results, since an increase in the number of scientific reports published in a particular taxonomy might not reflect a true increase in the incidence of disease; a particularly prolific author could bias the search results by turning up more cases of disease in a population than actually occurred; or a single disease event reported multiple times in different papers could create the impression that disease had suddenly increased. To normalize publication rates over time, Ward and Lafferty used a proportion of disease reports from a given population relative to the total number of reports in that group. To determine whether there was an "author effect," they removed the most prolific author in each taxonomic group and found that an author's abundant contributions did not skew the results. Finally, they confirmed that a single disease didn't bias their results by removing multiple reports of the same disease from the literature before analyzing the trends.

When they analyzed the searches without adjusting for the total number of reports published, Ward and Lafferty found that reports of disease increased for all groups. But when they analyzed the normalized results, they found that trends varied. While there was a clear increase in disease among turtles, corals, mammals, urchins, and mollusks, they found no significant trends for seagrasses, decapods, and sharks/rays. And they found that disease reports actually decreased for fishes. (One explanation for this decrease could be that drastic reductions in population density present fewer opportunities for transmitting infection.) Ward and Lafferty tested the soundness of this approach by using a disease (raccoon rabies) for which baseline data exist and showing that normalized reports of raccoon rabies increased since 1970, just as the disease increased from one case reported in Virginia in 1977 to an "epizootic" outbreak, affecting eight mid-Atlantic states and Washington, D.C., by 1992.

The pattern of increased reports, the authors propose, confirms scientists' perceptions about the rising distress of threatened populations and thus reflects a real underlying pattern in nature. The fact that disease did not increase in all taxonomic groups suggests that increases in disease are not simply the result of increased study and that certain stressors, such as global climate change, likely impact disease in complex ways. By demonstrating that an actual change in disease over time is accompanied by a corresponding change in published reports by scientists, Ward and Lafferty have created a powerful tool to help evaluate trends in disease in the absence of baseline data. It is only by understanding the dynamics of disease outbreaks that scientists can help develop sound methods to contain them.

Ward JR, Lafferty KD (2004) The elusive baseline of marine disease: Are diseases in ocean ecosystems increasing? DOI: 10.1371/journal.pbio.0020120



A dead gorgonian sea fan on a wall in Palau (Photograph, with permission, by Drew Harvell.)