

# Synopses of Research Articles

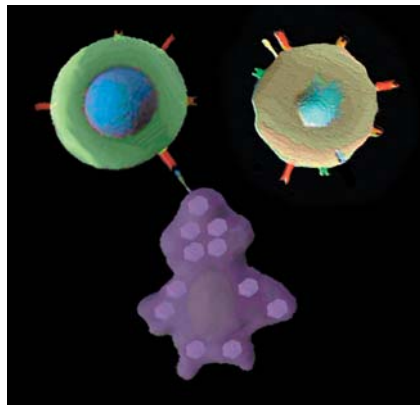
## T-Cell Differentiation and Progression of HIV Infection

Of the 300 or so viruses that cause disease in humans, HIV may have the greatest adaptive advantage. Like most persistent viruses—including the herpesviruses Epstein–Barr and cytomegalovirus (CMV)—HIV employs various strategies to counteract its host's response to infection. But HIV possesses a unique ability to sustain a progressive attack on the immune system—infesting the very cells that coordinate the immune response—leaving the body susceptible even to normally harmless microorganisms. It is these so-called opportunistic infections, rather than the human immunodeficiency virus itself, that makes HIV so deadly. The specific mechanisms that engineer this ongoing systemic attack have been the subject of intense research.

HIV targets white blood cells with protein surface receptors called CD4. These CD4, or helper, T-cells normally orchestrate the body's immune response by signaling killer T-cells (which are also called CD8 T-cells, after their CD8 surface receptors) and other immune cells to multiply and differentiate—that is, become specially equipped to recognize a particular pathogen, or antigen. At the onset of infection, the immune system appears to respond normally, with a strong attack led by HIV-specific CD8 T-cells that initially contain the virus. But as the infection progresses, CD4 counts drop and the body's ability to renew T-cells decreases while its proportion of “antigen-experienced” CD8 T-cells increases. While the biological effect of this hyperactivity is unclear, it is apparent that patients with elevated immune activity face a poor prognosis. Investigating the interaction among immune activation, CD8 T-cell differentiation, and HIV prognosis, Victor Appay and colleagues report that a close connection between elevated immune activation and elevated levels of highly differentiated T-cells may bring further insights into how HIV exhausts the immune system.

To examine the effect of elevated immune activation on T-cells, the researchers analyzed T-cells from a group

of HIV-infected individuals collected at two distinct points in time: at the onset of acute infection—which is characterized



**Two T-cells, one of which recognizes a target cell**

by vigorous HIV replication—and after treatment, when viral replication is suppressed. To explore the connection between T-cell differentiation and clinical status, the researchers analyzed the T-cells from a group of untreated infected individuals divided into three subsets based on stage of infection: acute infection, chronic infection without progression, and chronic infection with signs of progression.

During acute HIV infection, the vast majority (80%–90%) of the CD8 T-cell population was activated—not just the HIV-specific CD8 T-cells. Surprisingly, CD8 T-cells specific to the Epstein–Barr and CMV viruses showed significant activation levels during acute infection, suggesting that HIV may indirectly promote the replication of these viruses. When the researchers investigated the effects of this activation on T-cell differentiation, they found a correlation between increasing antigen concentrations and increasing CD8 T-cell activation and proliferation. And when Laura Papagno et al. analyzed the differentiation state of CD8 T-cells in individuals at different stages of infection, they found a progression in the proportion of highly differentiated CD8 T-cells associated with HIV disease progression.

These results, the researchers conclude, show that chronic overactivation of the immune system during HIV infection produces the large pool of highly

differentiated T-cells observed in HIV infection. T-cells go through various stages toward late differentiation, and it may be that the early-differentiated CD8 T-cells, which maintain the ability to proliferate, offer protective immunity. But highly differentiated cells, they propose, exhibit characteristics associated with “replicative senescence”—they are in effect old, worn-out cells that can no longer proliferate. Though replicative senescence is a natural process for most cells, in the context of HIV—in which infected individuals also lose the ability to replenish T-cells—it creates an aging population of T-cells that are less effective at fighting infection.

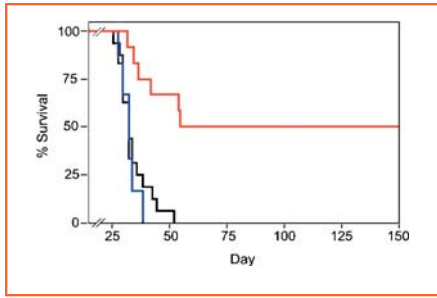
**Papagno L, Spina CA, Marchant A, Salio M, Rufer N, et al. (2004) Immune activation and CD8<sup>+</sup> T-cell differentiation towards senescence in HIV-1 infection. DOI: 10.1371/journal.pbio.0020020**

## Activating p53 in Cancer Cells with Protein Therapy Shows Preclinical Promise

Late-stage cancers are notoriously unresponsive to treatment, making certain hard-to-detect cancers particularly insidious. Ovarian cancer, for example, most often escapes diagnosis until the tumor has already metastasized. At this stage, ovarian cancer is classified as peritoneal carcinomatosis, a terminal condition characterized by widespread tumor growth throughout the peritoneum, the large serous membrane that lines the abdominal cavity, pelvis, and associated organs. Advanced cases of peritoneal carcinomatosis are largely resistant to chemotherapy and account for the bleak 15%–20% survival rate of ovarian cancer.

Biologists often view cancer as an evolutionary process in which cells that would normally cooperate with their neighbors begin to compete with them. Selective advantage for cancer cells often begins with mutations that inhibit tumor suppressor pathways. p53, like other tumor suppressor genes, arrests cell growth and induces apoptosis (programmed cell death) in response to cellular stress, such as chromosomal damage. Cells with p53 mutations often escape these constraints, leading to the uncontrolled growth characteristic of “immortal” cancer cells. Nearly all types of tumors have mutations in the p53 pathway. Treatments focused on restoring p53 function—which is likely





**TATp53C' treatment extends survival of mice**

developing targeted therapeutic strategies, Steven Dowdy and colleagues show that restoring p53 protein function in tumor cells not only dramatically increases lifespan in mice but also eliminates disease.

While past efforts to restore tumor suppressor function in cancer cells have focused on gene therapy, Dowdy and colleagues introduced modified p53 peptides, or protein fragments, into cancer cells. p53 works as a “transcriptional” activator that binds to specific sequences of DNA and triggers apoptosis in response to DNA damage. Its biological function flows from this binding ability. One region of this large protein, called the C-terminal domain, facilitates effective binding. In cancer cells, synthesized peptides (called p53C') derived from this region can induce apoptosis by activating p53—which is normally present in low levels in a biologically inactive form—and restoring function to p53 proteins with DNA-binding mutations.

To get p53C' peptides into cancer cells, Eric Snyder et al. used a technique pioneered by Dowdy that delivers large proteins into the cell interior. Since the cell membrane normally limits passage to only small molecules (larger molecules generally enter through surface receptors), this is no small feat. The technique exploits the ability of a small peptide region from the HIV TAT protein to smuggle macromolecules through cell membranes that normally prohibit entry to such large molecules. After synthesizing a structurally modified form of p53C' less prone to degradation, the researchers first confirmed that the peptide was functional and then that it activates p53-specific genes in tumor cells, but not in normal cells. Testing the effectiveness of the peptide therapy on mouse strains used to model human metastatic disease, they found that mice treated with the TATp53C' peptide showed a significant reduction in tumor growth and lived six times longer than both mice treated with a control peptide and untreated mice, with some mice remaining disease-free more than 200 days after treatment.

This macromolecular delivery approach, Snyder et al. argue, works with greater specificity and avoids the tumor-generated neutralizing effects observed in small molecule strategies. Because a mutation in the p53 gene is one of the most common events in the development of cancer, these results could have implications for a wide variety of cancers. And by working with mouse models that approximate the physiological burdens metastatic cancer imposes on humans, Dowdy's team demonstrates the promise of developing targeted “intracellular biologic” therapeutics that treat the systemic pathology of cancer—inhibiting tumor growth as well as alleviating the lethal complications associated with the disease.

Snyder EL, Meade BR, Saenz CC, Dowdy SF (2004) Treatment of terminal peritoneal carcinomatosis by a transducible p53-activating peptide. DOI: 10.1371/journal.pbio.0020036

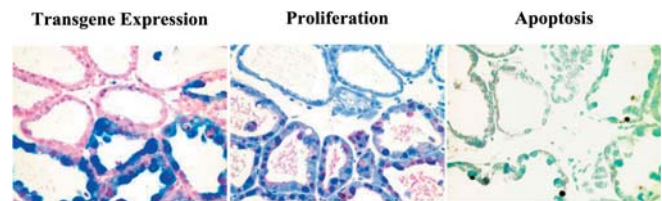
to be defective only in cancer cells—should prove more effective than chemotherapies, which indiscriminately kill all dividing cells, healthy or cancerous.

With the goal of

## A New Breast Cancer Model

Thanks to the tools of molecular biology, our understanding of the 100-plus diseases known collectively as cancer has increased dramatically over the past decades. While each of these cancers exhibits unique characteristics reflecting the particular cell or tissue it springs from, the disease follows a similar arc in nearly all its forms. Cancer is a multistep disease that begins when genetic damage—initiated by a multitude of agents—unleashes a single cell from the normal constraints on cellular proliferation. This single transformed cell generates a colony of similarly abnormal progeny that can take decades to develop into malignancies.

While events that stimulate uncontrolled cell division can promote cancer, mutations in tumor suppressor genes figure prominently in tumor progression. Disruptions in the pRb (retinoblastoma 1) tumor suppressor, for example, are often seen early in cancer development, sensitizing cells to tumorigenesis. pRb, along with other “pocket proteins”—so-called because they share an amino acid domain called the Rb pocket—regulate cell cycle progression, apoptosis (programmed cell death), and cellular differentiation. Some tumor suppressors, such as p53, can trigger apoptosis, ultimately sacrificing cells that have sustained DNA damage or other types of cellular stress.



**Transgene expression is associated with increased cell proliferation and cell death (apoptosis)**

Mutations in both the pRb and p53 tumor suppressor pathways are commonly seen in human cancers, though their interactions appear to vary depending on the tissue. In mouse brain epithelial cells, for example, loss of p53 function coupled with loss of pRb results in reduced apoptosis and increased tumor growth, while p53 loss in mouse brain astrocytes (cells that support neurons) does not affect tumor growth. Building on this work, Terry Van Dyke and colleagues report that loss of the pRb tumor suppressor in mammary tissue has the same effect—predisposition to tumor formation—seen in these other cell types. Despite the different environment inherent in each cell type, the initial events following loss of the pRb pathway were the same: increased proliferation and apoptosis, followed by tumorigenesis. But, surprisingly, pRb and p53 interactions varied in different cell types.

Like most cancers, mammary gland cancer has a long latency period, prompting the researchers to ask what events engineer tumor progression. To investigate the relative contribution of pRb and p53 in tumorigenesis, the researchers generated a novel mouse model with a dysfunctional pRb pathway and various levels of p53 function in several cell types. This is a significant achievement in itself, as many agents that inactivate the pRb pathway also disrupt the p53 pathway. pRb inactivation, they show, causes abnormalities in mammary cell proliferation, apoptosis, and tissue morphology. In these mammary-specific pRb-deficient mice, p53 was responsible for most of the apoptotic response—decreased levels of p53 resulted in reduced

apoptosis and accelerated tumorigenesis, but had no effect on proliferation. Interestingly, in other mouse models where aberrant proliferation is caused by disabling other pathways, loss of p53 was associated with increased proliferation—rather than reduced apoptosis—and early tumor formation. And while p53 is the main effector of apoptosis in brain and mammary epithelial cells, this is not the case in all tissues: in astrocytes, for example, the tumor suppressor Pten regulates apoptosis in response to pRb inactivation. Together these results indicate that specific cellular responses

to a cancer-causing stimulus vary depending on the nature of the initial genetic injury and the cell type and that pRb and p53 interact in different ways in different tissues. And p53, it appears, contributes to tumor suppression—and thus progression—through multiple mechanisms.

By creating a mouse model that disentangles the pRb and p53 pathways, Van Dyke and colleagues have added a valuable resource for studying breast cancer. This model, they propose, will facilitate further investigations into the relative contributions of these

overlapping pathways to cancer progression. What's more, the model offers a vehicle for examining how pRb interacts with other breast cancer mutations, like the inherited mutations in the human *BRCA1* and *BRCA2* genes, to shed light on the complex series of events that ultimately cause breast cancer.

**Simin K, Wu H, Lu L, Pinkel D, Albertson D, et al. (2004) pRb inactivation in mammary cells reveals common mechanisms for tumor initiation and progression in divergent epithelia. DOI: 10.1371/journal.pbio.0020022**

## Gene Expression Signature of a Fibroblast Serum Response Predicts Cancer Progression

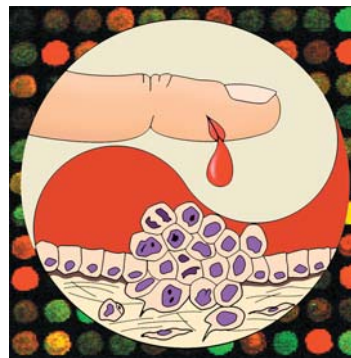
The idea that cancer cells go through a fateful transition that turns them into fast-growing, invasive, metastasizing tumors first surfaced in the early 1970s. During this conversion, blood vessels form around the tumor, providing a dedicated supply of blood to fuel the tumor's aggressive behavior. By the mid-1980s histological analysis revealed a similarity between the tumor "microenvironment" and that of a healing wound, prompting Harvard pathologist Harold Dvorak to describe cancer as a wound that does not heal. When the body sustains a wound, it coordinates an emergency response defined by rapid cell proliferation, invasion and "remodeling" of connective tissues and extracellular matrix (the network of proteins and molecules around cells), cell migration, and blood vessel formation (angiogenesis). These processes, which are restorative in normal wound healing, may promote cancer by supporting tumor formation, invasion, and metastasis. With no systematic method to measure the "wound-like" features in cancer, however, scientists have no way to evaluate the risk that a wound-healing genetic program may pose in cancer progression.

A molecular understanding of the wound-healing process and its connection to cancer would provide insight into the nature of these similarities and perhaps provide molecular indicators of tumor progression. In an effort to create a framework for evaluating this relationship, Howard Chang and his colleagues at Stanford University developed a model to predict cancer progression based on the gene expression profile of a cellular response to serum in cell culture.

Part of the problem with evaluating the physiological status of a tumor based on its genetic profile is that current techniques indicate only the expression, not the effect, of genes. To develop a strategy for interpreting biological outcomes from a gene expression profile, Brown's team modeled a physiological process by exposing cultured fibroblasts to serum—the soluble fraction of coagulated blood—and tracking gene expression. Serum is encountered in the body where blood leaks out of blood vessels (in essence, all the sites of injury) and is thought to be a major initiator of the wound response. Fibroblasts exist in the connective tissue of epithelial organs (which include the digestive tract, lungs, and mammary glands) and contribute to organ development, wound healing, inflammation, and a condition called fibrosis. (Fibrosis involves the same type of extracellular matrix remodeling seen in wound healing and cancer.) And fibroblasts can promote tumor formation and metastasis when mixed with epithelial cancer cells.

Though fibroblasts from different sites in the body differ in

their properties and gene expression profiles, Chang et al. found that they share a common expression pattern in response to serum. From this expression profile, the researchers identified a core group of genes—a genetic signature—associated with a serum response. Because many of the genes in the signature were known to be involved in various wound-healing processes—such as matrix remodeling, cell motility, and



**Genomics predicts tumor behavior**

angiogenesis—Chang et al. used this signature as a surrogate marker to measure how much tumors may be like wounds. When they compared the wound-like genetic signature with the expression profiles of various clinical tumor samples, they found the signature was always present in certain cancers—prostate and liver-cell carcinomas—and

occurred variably in others—breast, lung, and gastric carcinomas. In each of these three latter types of tumors, patients with tumors carrying the serum-activated wound-like genetic signature had a significantly increased risk of metastasis and death compared to patients with tumors that lacked the signature. Therefore, Chang et al. conclude that a wound-like phenotype is a general risk factor for metastasis and the aggressive behavior in many of the most common cancers.

These results reveal a robust and useful similarity between the molecular programs in normal wound healing and tumor progression and metastasis. Although Chang et al. point out that their results do not indicate whether this fibroblast "fingerprint" is merely a marker for cancer progression or plays a role in orchestrating this pathway, they conclude that the genetic program activated in response to serum also contributes to tumor invasion and metastasis. This serum-response expression profile, the authors propose, provides a valuable new tool for predicting tumor behavior and determining a patient's prognosis.

**Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, et al. (2004) Gene expression signature of fibroblast serum response predicts human cancer progression: Similarities between tumors and wounds. DOI: 10.1371/journal.pbio.0020007**

## Diverse Signals Establish the Left-Right Body Axis

Most animals (including humans) show a high level of bilateral symmetry: on the surface, the right side of our body resembles the left. A closer and deeper look, however, reveals an underlying asymmetry. The heart, for example, is on the left side in most humans, and the liver on the right. This left-right asymmetry develops early on in the embryo, and research in the past few years has revealed some of the molecular and cellular mechanisms that establish the left-right axis, which conveys positional information to cells in the growing embryo. We know that the formation of the axis relies on “crosstalk” between cells, which involves long-range signaling molecules (or ligands) and cell-surface receptors on cells that receive the signal.

The molecules involved in the formation of the left-right axis during embryogenesis, along with their functions, are conserved among vertebrates. They include members of the Transforming Growth Factor beta (TGF- $\beta$ ) family—such as the agonists (or ligands) Nodal, Vg1/GDF, and activin, and the antagonist (a molecule that interferes with agonist/ligands) Lefty—on the signaling side and members of the EGF-

CFC family—such as the activin receptor and its coreceptors—on the receiving side. The EGF-CFC proteins play important roles in early vertebrate embryogenesis; mutations in these genes in the zebrafish (and mouse) result in a range of developmental defects, including problems in left-right axis specification. While ligand-stimulation of the activin receptor by Nodal and Vg1/GDF requires the EGF-CFC coreceptors, activin can activate the activin pathway without a coreceptor. Lefty—being an antagonist—can block activation of the activin receptor, though it is not clear how.

Through genetic and biochemical studies in zebrafish and frog embryos, Simon Cheng, Alex Schier, and colleagues have now clarified a piece of this very complex signaling puzzle by demonstrating that Lefty inhibits a subset of TGF- $\beta$  signals—Nodal and Vg1/GDF but not activin—by blocking EGF-CFC coreceptors. They went on to show that a short, specific region of the signal molecules—accounting for less than 4% of the entire protein—determines whether the signals activate the activin receptor in an EGF-CFC coreceptor-dependent or -independent fashion



**Zebrafish embryo heart loops correctly in wild-type, incorrectly in mutant**

and therefore governs susceptibility to Lefty. These findings suggest that subtle sequence differences between related signals can dramatically influence their function.

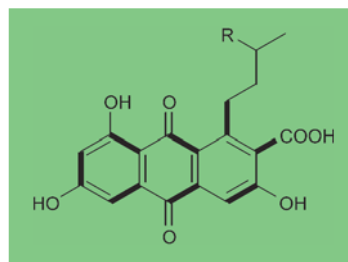
Gene families are thought to arise from gene duplications, and the studies described here illustrate how members of the same gene families can gain diverse roles by specific interactions with coreceptors and antagonists. Additional studies will be necessary to reveal the structural basis for the observed diversity.

**Cheng SK, Olale F, Brivanlou AH, Schier AF (2004) Lefty blocks a subset of TGF $\beta$  signals by antagonizing EGF-CFC coreceptors. DOI: 10.1371/journal.pbio.0020030**

## Engineering Bacteria to Make “Unnatural” Natural Drugs

Faced with new and ongoing threats to public health, researchers are becoming increasingly resourceful in their quest to discover new drugs. Drug researchers have long looked to living organisms for inspiration, either mimicking or extracting chemical formulas from naturally occurring compounds. Bacteria and fungi, for example, produce a wide range of compounds—some of which give them a selective advantage in their own environments—that provide important pharmaceutical activities. One class of these natural compounds are the polyketides, which make up a large portion of the antibiotics (including erythromycin and tetracycline) and antitumor drugs (such as doxorubicin and epothilone) that have been isolated from various microorganisms.

Polyketides are synthesized by bacteria and fungi by the appropriately named polyketide synthases (PKSs). PKSs can be thought of as large molecular factories containing a series of enzymes working on an assembly line: each enzyme in the line adds molecules to a primer, or starter, unit—which is usually an acetate molecule—and then hands off the growing chain to the next enzyme. The specific enzymes set all the characteristics of the polyketide, including the chain length, the building blocks used, and the branching pattern of the molecules. Although microorganisms generate polyketides with a variety of characteristics, one goal of drug discovery research is to increase this diversity even further—a larger pool of polyketides promises more drugs with enhanced pharmaceutical applications.



**A synthetic polyketide**

Early attempts at creating artificial polyketides focused on altering the functional characteristics of naturally occurring polyketides—the length of the chain, the building blocks, and the patterns of the branches. Chaitan Khosla and colleagues have taken this approach one very large step further. Rather than changing the machinery to modify the growing structure

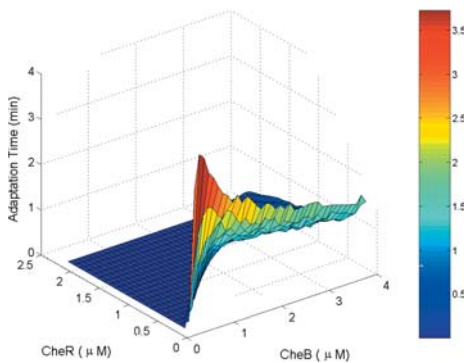
of a polyketide, they engineered bacteria to use an alternative, nonacetate primer molecule. This has important practical implications because some medicinally significant compounds do not use the usual acetate primer unit. By dissecting out the specificities of the “starter” and longer, multiunit “elongation” PKS enzymes and by mixing and matching modules, they have produced novel polyketide analogs (in this case, of anthraquinone) with more effective medically relevant properties. One of the compounds they engineered shows enhanced efficacy in blocking the growth of breast cancer cells that depend on the activity of the estrogen receptor, while a second polyketide inhibits an enzyme linked to adult-onset diabetes, demonstrating just two possible new therapeutic applications for synthesized polyketides. But, as the authors propose, this method promises to reveal new pharmaceutical agents that haven’t even been discovered yet.

**Tang Y, Lee TS, Khosla C (2004) Engineered biosynthesis of regioselectively modified aromatic polyketides using bimodular polyketide synthases. DOI: 10.1371/journal.pbio.0020031**

## Comparing the Networks that Power Bacterial Chemotaxis

When we think of foraging for food, we usually imagine animals wandering in the woods, poking behind bushes and trees, trying to find something tasty. Amazingly, even single-cell bacteria display a simplified version of this behavior. Many species of bacteria can respond to chemical or nutritional cues (chemoattractants) in the environment by moving toward locations with more favorable conditions, a process known as chemotaxis. The bacteria adjust their movements by rotating threadlike projections called flagella either clockwise or counterclockwise; these adjustments are made by a network of proteins in response to chemoattractants. Chemotaxis has been identified in many bacterial species, but two of the best-studied examples are in *Escherichia coli* and *Bacillus subtilis*. Computer modeling of chemotaxis in these species now reveals some important differences in the network architecture that controls this complex behavior.

Most of the proteins involved in chemotaxis in *E. coli* and *B. subtilis* have been identified and well studied, but much remains to be learned about this biological process. As scientists have begun to understand how the proteins work together, they're discovering a network of interactions that operates a bit like an electronic circuit. Researchers have found that using the circuit as a model for protein networks has helped them to understand how complex system properties arise from seemingly simple



Modeling chemotaxis

interactions between proteins. These properties can be explored with the aid of computer simulations, whereby researchers can rapidly test a given system under many different situations and can tweak the properties of the proteins and their connections.

The team, led by Adam Arkin of the University of California at Berkeley, has compared the system level properties of chemotaxis in the two bacterial species *E. coli* and *B. subtilis*. Not surprisingly, the proteins involved in the signaling pathway are conserved—that is, they have changed very little since they first evolved—even though

these species are evolutionarily very distant. In many cases, a gene from one species can even substitute for the ortholog (a conserved gene that retains the same function even though two species have diverged) in the other. Despite these similarities, however, disrupting the function of orthologous genes in these two species often has different, even opposite, effects. This is surprising, especially given that the chemotactic behaviors of *E. coli* and *B. subtilis* are almost identical. In order to understand this puzzling observation, the researchers constructed a network model of the chemotaxis system in *B. subtilis* and used simulations to understand how the network properties differ from those of existing models of *E. coli* chemotaxis.

The group found that despite the similarities in proteins and the nearly identical behavior between the two species, the mechanisms underlying the behavior are quite distinctive. When comparing the system properties of these two bacterial systems, the researchers also made an unusual observation. Though the two “circuits” have different wiring, the system properties underlying the behavior, called the control strategy, are very similar. The two species of bacteria therefore achieve the same chemotaxis behavior by using similar proteins, but in different ways.

Arkin and colleagues draw two important conclusions from these results. First, these two systems have conserved proteins, but the proteins are wired together differently. This means that wiring of signaling networks cannot be inferred simply by identifying the conserved proteins in the network. Second, in these systems, conserved proteins use different mechanisms to accomplish the same overall control strategy. This raises the question of how such systems evolve. The authors suggest that the control strategy itself may be an evolutionarily conserved property. These conclusions will be important to keep in mind as researchers examine these systems in more detail and begin to examine more complex systems as well.

Rao CV, Kirby JR, Arkin AP (2004) Design and diversity in bacterial chemotaxis: A comparative study in *Escherichia coli* and *Bacillus subtilis*. DOI: 10.1371/journal.pbio.0020049

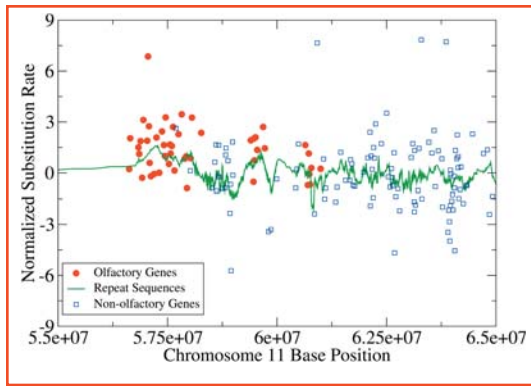


## Mutation Rates and Gene Location: Some Like It Hot

The growing library of sequenced genomes is challenging scientists to extract new biological meaning from DNA sequences. Comparative analysis of the mouse and human genome, for example, has already revealed that mutation rates in the 3 billion base pairs of the human genome vary considerably. What accounts for this regional disparity, however, is unclear. Mutations—substitutions in the nucleotide bases of DNA—produce variation in the genome. In classical evolutionary theory, natural selection drives evolutionary change by determining which of these mutations live on in the next generation or die with the organism. Mutations can be neutral, harmful, or beneficial, though the neutral theory of molecular evolution predicts that most mutations are “nearly” neutral or only slightly deleterious, while beneficial mutations—which confer a survival advantage on an organism and, if it reproduces, on its progeny—are quite rare. As a whole, mutations occur at the rate of approximately five substitutions per billion nucleotide sites per year.

There are many types of neutral mutations—that is, mutations that have no effect on function. DNA base substitutions that lie outside of gene-coding regions or occur within introns (regions that are excised before being translated into a protein sequence) can fall into this category. Neutral mutations can also occur within gene-coding regions. For example, there are many instances where more than one codon—say, CUU, CUC, CUA, CUG—specify the same amino acid—in this case, leucine. Since these mutations can be used to gauge the neutral mutation rate of a region in the genome, they can be used to analyze the relationship between local mutation rates and gene location. Correlating gene mutation rates with their location in the genome, Jeffrey Chuang and Hao Li not only confirm that regional mutation rates indeed exist, but also calculate the size of these regions. Strikingly, certain classes of genes tend to congregate in mutational “hot spots”—regions with high mutation rates—while other types of genes gravitate toward “cold spots”—regions with relatively low mutation rates.

Chuang and Li first determined whether mutation rates have regional



### Olfactory genes lie in a mutational “hot spot”

biases—that is, whether the frequency and distribution of mutations follow a distinct pattern along the genome. The researchers calculated the substitution rates of neutral mutations in nearly 15,000 orthologous mouse and human genes—orthologous genes are genes that have evolved from a common ancestor without diverging in biological function—and found that mutation rates were in fact skewed toward either high or low rates. Mutation rate analysis of the orthologs’ neighbors revealed rates similarly skewed toward high or low substitutions, indicating that the

for example, one would expect to find genes that would benefit from high rates of mutation, which would in turn facilitate flexible responses to constantly changing environmental stimuli. Likewise, one would expect genes in cold regions to need protection from potentially deleterious mutations. And that’s just what Chuang and Li found. Overall, genes in hot regions code for proteins involved in cell signaling, such as olfactory receptors, G-protein coupled receptors, membrane proteins, and immune response proteins—being in an area subject to high mutation rates means these genes

region itself, rather than a particular gene, is prone to these differential rates. These regions, Chuang and Li report, were either one megabase or ten megabases long, affecting up to roughly 100 genes.

But the question remained: Does the organism take advantage of these mutational hot and cold spots? If there is an adaptive advantage, gene families should occur in an appropriate mutational zone. In mutational hot spots,

can evolve quickly enough to adapt to constantly changing stimuli. Cold-region genes code for “housekeeping” proteins involved in core cellular processes, like transcription regulation and protein modification—these genes tend to be highly conserved, changing very little since they first evolved.

Thus, it appears that natural selection may also operate at the level of gene location, relegating genes to different mutational genomic niches according to their function. While Chuang and Li explore possible mechanisms to account for these genomic niches—such as gene duplication and gene transposition—they argue that the selective pressures that influence gene location are the same that influence mutations in genes. By calculating the sizes of these mutational hot and cold regions, the researchers lay the groundwork for investigating genetic mechanisms that operate on these scales. And by showing that location matters, they have revealed a new force in genome evolution.

**Chuang JH, Li H (2004) Functional bias and spatial organization of genes in mutational hot and cold regions in the human genome. DOI: 10.1371/journal.pbio.0020029**

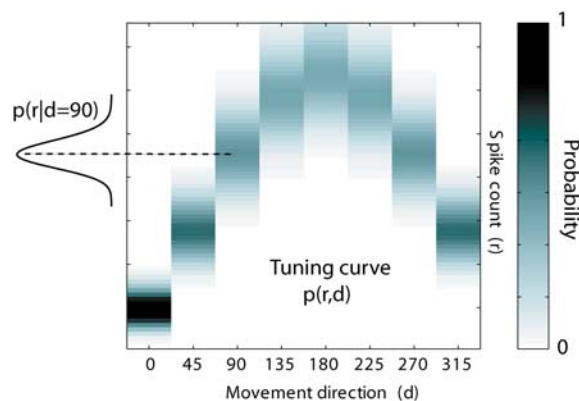
## When Monkeys Learn Directional Tasks, Neurons Learn Too

If you’ve ever hit a patch of ice on the road that sent your car swerving left while you resolutely—and futilely—steered right to get back in your lane, you’ve experienced what neuroscientists call a “visuomotor rotation task.” On a dry road, your response would have been appropriate. But under icy conditions, the same sensory cue produces a decidedly negative result: a car fishtailing out of control. While you’re figuring out what movements will straighten out the car, the neurons in your primary motor cortex—the region of the brain responsible for movement—are taking notes. Chances are, your next icy encounter was less dramatic. But how does your brain learn to produce a different movement in response to the same visual cue?

Neuroscientists investigate such questions by recording and analyzing the electrical activity of neurons during learning and performance of new sensory-motor transformations. Such studies, for example, show that populations of neurons in different brain areas map sensory cues and desired arm motion by creating an internal representation of the corresponding sensory and motor coordinates in a way that allows flexible responses to changing conditions. In previous studies, Rony Paz and Eilon Vaadia, of The Hebrew University in Israel, found that neurons in the primary motor cortex that fire before monkeys move their arm in a particular direction have higher firing rates after the monkey learns to dissociate the arm direction from the cursor direction (an indicator of visual feedback). Interestingly, changes in activity preferentially occurred in a subset of neurons that were already tuned (that is, maximally activated during movement) to the direction experienced while learning.

While many studies indicate that learning new tasks can

generate specific changes in brain activity, it had not been clear how or if such changes improve the internal representation inside the brain. Specifically, is the neuronal code any “better” after learning? Now Paz and Vaadia show that while these neurons are firing at higher rates they are also transmitting more information about specific task parameters.



### Mutual information between neuronal activity and direction of movement

Paz and Vaadia trained two rhesus monkeys to learn various visual-motor tasks—which involved operating a joystick to move a cursor on a screen—and then changed the relationship between the visual feedback (the cursor) and hand movement. Using information-theory analysis—which measures the amount of information that single neurons can tell about the

movement—they were able to correlate neuron activity with direction of movement and, conversely, distinguish differences between directions based on neuron activity. Their analysis revealed that the neurons transmit more information about the direction of movement after the monkeys learn a task. To figure out what aspect of neuron activity conveys this improved information, Paz and Vaadia examined two features of neuron signaling—response variability and directional sensitivity—which they reasoned might plausibly accomplish this. Increased

information content after learning a task, they found, corresponded to sensitivity to a single direction, and neurons attuned to that direction contributed to the increase.

These findings suggest that subsets of directionally sensitive neurons increase their firing rates to more finely tune their sensitivity to that direction. By successfully reconstructing the movement direction from the neuron signals captured after learning a task, Paz and Vaadia also demonstrate that the observed learning improvement can be extracted to predict behavior. The authors argue that their results suggest a

close association between properties of neurons—such as directional tuning of cells—and learning a skill that is focused on the same parameter—in this case, direction. Together with results from visual and auditory areas, they propose that similar mechanisms may control the interplay between neurons and learning throughout the central nervous system.

**Paz R, Vaadia E (2004) Learning-induced improvement in encoding and decoding of specific movement directions by neurons in the primary motor cortex. DOI: 10.1371/journal.pbio.0020045**

## Learning to Discern Images Modifies Neural Activity

The primate brain processes a remarkably diverse array of visual cues to recognize objects in dynamic settings crammed with unfamiliar objects. Not surprisingly, repeated viewing aids recognition, but how the brain orchestrates this experience-driven improvement is unclear. Visual input to the brain travels from the eye to the primary visual cortex (V1), at the back of the brain. From there, signals are sent to nearby extrastriate cortical areas, which process “early” visual cues. Both the “lower level” extrastriate cortex and “higher level” inferior temporal (IT) cortex are important for object recognition in primates. In monkeys and humans, lesions in the IT cortex severely affect the ability to recognize objects.

In these higher-level cortical regions, neurons carry more information about an object after subjects learn to recognize that

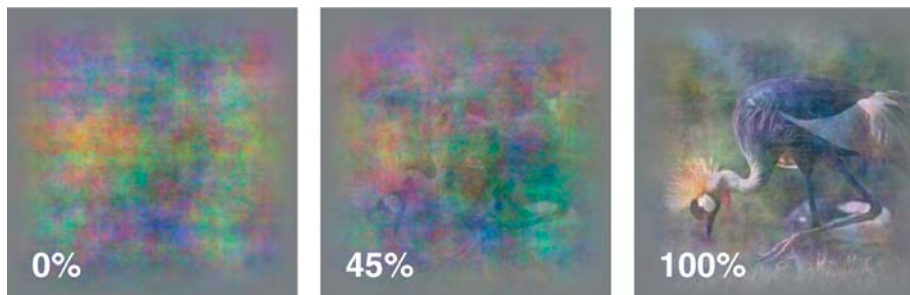
object. This modified neural activity is thought to reflect internal representations of specific aspects of the learned task—such as learned recognition of three-dimensional objects—and these representations often remain stable even though certain features of the visual stimulus—such as size or image degradation—change. With recent evidence suggesting that lower level brain regions like the primary visual cortex are also capable of learning-related modifications, it appears that both early and higher brain areas of the “ventral visual stream” benefit from learning. It is not clear, however, how learning modifies these discrete brain regions to coordinate this processing.

By training monkeys to recognize degraded images, Gregor Rainer, Han Lee, and Nikos Logothetis of the Max Planck Institute for Biological Cybernetics in Germany have identified a subset of neurons that compensate for indistinct visual inputs by coordinating disparate regions in the brain. The monkeys’ improved performance, they propose, stems from the informational enrichment of a subset of lower level neurons. Along with an increase in learning-induced firing activity, V4

neurons—extrastriate cortical neurons associated with detecting visual input of intermediate complexity—encode more information about relevant details to resolve indeterminate visual cues. V4 neurons likely interact with higher cortical levels to help the monkeys interpret the degraded indeterminate images as something recognizable.

The researchers presented the monkeys with different “natural” images, including pictures of birds and humans, then subjected the images to different levels of “stimulus degradation”—making them harder to read by adding varying

amounts of visual noise. Using this approach, the researchers could record the activity of the V4 neurons as the monkeys were presented with the different images. The monkeys viewed a sample image and then



**Monkeys can learn to recognize degraded images**

signaled whether a second image, presented after a brief delay, was a match or not. When Rainer et al. analyzed the activity of the V4 neurons associated with the different images, they found there was no significant change in the activity or information conveyed by V4 neurons associated with novel or undegraded familiar images. On the other hand, learning not only significantly improved the monkeys’ ability to recognize degraded stimuli but also increased both the activity and informational encoding of the V4 neurons.

But how did individual V4 neurons facilitate this enhanced ability to recognize degraded stimuli? After identifying a subset of neurons that showed enriched neural activity in response to degraded or indeterminate stimuli, the researchers studied the monkeys’ eye movements to determine any behaviors that might explain why monkeys performed better with familiar degraded stimuli. They mapped the monkeys’ eye movements while allowing them to freely view the different familiar and novel images—but this time with just two coherence levels (undegraded and 45% coherent). There was substantially more

overlap, in terms of where the monkeys looked for the 45% and 100% coherent images after learning. This suggests that monkeys learned to focus their attention on particular salient features, and were thus better able to identify degraded versions of these images.

Neurons in the V4 area appear to be recruited to distinguish the relevant visual signal from the visual noise, and thus play a critical role in resolving indeterminate stimuli when salient features are present. These results, together with previous studies showing the sensitivity of prefrontal cortex neurons to novel stimuli, indicate that the prefrontal cortex processes novel

stimuli while the V4-rich extrastriate visual areas convey details about hard to decipher images. It may be that as the V4 neurons refine their competence through learning, they also support the ability of the prefrontal cortex to process different but similar visual cues. Vision is a dynamic process, Rainer et al. conclude, characterized by ongoing interactions between stimulus-driven brain regions and feedback from higher-order cognitive regions.

**Rainer G, Lee H, Logothetis N (2004) The effect of learning on the function of monkey extrastriate visual cortex. DOI: 10.1371/journal.pbio.0020044**

