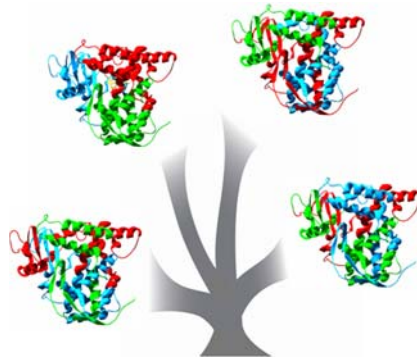


A Smart Mutation Scheme Produces Hundreds of Functional Proteins

Richard Robinson | DOI: 10.1371/journal.pbio.0040136

A protein's structure dictates its function, and one of the most direct and powerful ways to explore a protein's function is by modifying its structure. Such an exploration is carried out naturally every time a protein's gene is mutated, and the same process can be mimicked in the lab. Unfortunately, approaches that introduce random mutations frequently disrupt the interactions that keep a protein properly folded, rendering the mutant entirely functionless and the experimental results largely uninformative about the contributions made by specific amino acids to overall function. In a new study, Christopher Otey, Frances Arnold, and colleagues use recombination guided by structural modeling to efficiently generate a family of thousands of properly folded mutants of a protein, and reveal previously unknown influences on the protein's function.

The authors studied versions of the protein cytochrome P450—a diverse protein family whose members govern a host of cell reactions, including detoxifying drugs and aiding construction of a wide variety of complex molecules. Each cytochrome P450 has nestled within it a heme group, a two-dimensional cage for an iron atom that is also found at the heart of hemoglobin. To generate the new cytochromes, they used a structural analysis tool called SCHEMA, which identifies how to divide multiple cytochromes into smaller “blocks.” Reassembling these blocks creates chimeras that have a good chance of being functional, but which, at the same time, have large numbers of mutations (up to 109) compared with the parent sequences. Otey et al. modeled the consequences of millions of such mutations, and chose a set of blocks that, according to the analysis, retained the greatest number of contacts between amino acids in the protein, reasoning that this would increase the likelihood of proper folding. Moving from computer to Petri dish, they then generated a set of over 6,000 new genes and expressed them in bacteria.



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The creation of an artificial family of thousands of new proteins by shuffling protein building blocks allows us to probe protein structure and function, free from the filtering effects of natural selection.

From the bacterial colonies, each with a unique cytochrome P450, they randomly chose almost 1,000 for detailed analysis. In about half of these, the protein folded properly and bound its heme group, despite differing from the naturally occurring proteins at 70 out of about 460 amino acid positions on average. Of those that folded correctly, about three-quarters were also functionally intact, able to catalyze reactions similar to those of the native

proteins. Detailed analysis revealed the contribution to catalytic activity of a previously unappreciated amino acid near the enzyme's active site. Some of the mutants were more resistant to heat degradation than the originals, indicating the potential for creating novel and possibly useful new variants using this method.

The mutation method used here increased the yield of properly folded cytochromes by 10,000-fold over entirely random mutation techniques, and in one fell swoop nearly doubled the number of extant functional cytochromes P450. The results of this study will be useful for further structural analysis of the cytochrome P450 family, and the SCHEMA method for generating structurally intact mutants is likely to be applied to other protein families as well, both to tease out their structural secrets and perhaps to generate proteins with new properties that could be exploited for commercial or medical applications.

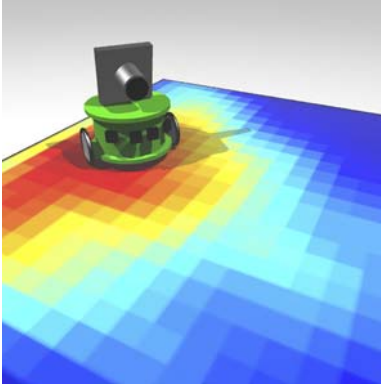
Otey CR, Landwehr M, Endelman JB, Hiraga K, Bloom JD, et al. (2006) Structure-guided recombination creates an artificial family of cytochromes P450. DOI: 10.1371/journal.pbio.0040112

Just a Few Computational Principles Generate a Realistic Model of the Brain's Visual System

Liza Gross | DOI: 10.1371/journal.pbio.0040161

In the 1950s science-fiction cult classic *Forbidden Planet*, Robby the Robot talks, cleans, learns new tasks, and understands the commands of his masters. Scientists are not at the point where they can produce Robby knockoffs with computer-driven cognition, but they are learning to use robots to probe the structure and function of the human brain. In a new study, Reto Wyss, Peter König, and Paul Verschure use a data-collecting, ambulatory robot to test a model of visual perception based on two computational functions.

When exposed to visual stimuli, neurons in the visual cortex respond to salient properties in the visual environment to create an internal representation of the world. Visual inputs are collected by the retina, sent to the thalamus, and then travel through a series of subcortical and cortical structures before reaching higher cognitive structures in the ventral visual system, including the hippocampus. In this “feed-forward” model of visual processing, neurons at different points in this visual-processing hierarchy learn to acquire increasingly



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A mobile robot helped test a model of the ventral visual system based on two computational principles.

complex, refined, and specific responses to these signals—even though they inhabit anatomically similar structures. There also seems to be some crossover in job duties, with evidence that the functions of specialized areas can be assumed by other regions. How the different regions of the brain acquire their specialized functions remains an open question. Is specialization an inherent trait, with each cortical region following unique computational principles? Or does each region follow the same principles and learn its specialized tasks based on its different position and input?

Theoretical neuroscientists investigate such questions by creating models to simulate the computational tasks performed by different brain structures. Such approaches have identified statistical measures called “objective functions” that can describe the computational principles of the

primary visual cortex, which processes signals from the retina. For example, a statistical property that optimizes sparse representations corresponds to neurons called simple cells, while optimally stable representations correspond to complex cells. Wyss et al. asked whether objective functions could also describe the computational principles that govern the integration of visual stimuli across cortical regions.

To investigate this question, the researchers used a mobile robot programmed to navigate its environment while collecting visual inputs through a camera embedded in its circuitry. The camera provides ongoing inputs to the researcher’s visual system model, which includes connections both within and between five computational units in the visual hierarchy. The model includes an unsupervised learning algorithm to optimize the stability of visual representations in feed-forward connections in conjunction with ongoing independent neuron interactions within each level—representing local memory—simulating stimulus-driven learning. The feed-forward connections also show increasing convergence, akin to that reported in the primate visual pathway.

How did the model respond to the robot-collected input? After nearly three days, all the computational levels achieved stable representations, with higher levels reaching stability only after lower levels had done so. At this point, the computational units exhibited selectivity in their response properties. Lower-level units responded to features visible from

many different positions within the robot’s environment and had large responsive areas that depended on the robot’s orientation. Intermediate units responded to landmarks—particular views from a small region—and were highly selective for the robot’s orientation. The higher units learned to link nearby landmarks, relying on small responsive regions. And the highest unit grouped these landmarks into a more complex system for representing external space—a place field—which was highly dependent on the robot’s position.

The researchers used the responses of the different levels to reconstruct the position of the robot, and found that responses from the highest computational unit produced the most accurate reconstruction—in keeping with reconstructions based on the responses of rat hippocampal place cells.

These results indicate that just a few general computational principles, temporal stability and local memory, can produce specialized functions in different cortical areas. Specialization is not an intrinsic feature of these cortical areas but comes from the complex visual properties of the environment. This model of functional organization likely applies to other sensory systems, Wyss et al. conclude. If it turns out that just a few computational principles underlie higher cognitive functions as well, a real-life Robby may not be so far-fetched after all.

Wyss R, König P, Verschure PFMJ (2006) A model of the ventral visual system based on temporal stability and local memory. DOI: 10.1371/journal.pbio.0040120

How an Aggressive Weedy Invader Displaces Native Trees

Liza Gross | DOI: 10.1371/journal.pbio.0040173

Humans have never been known to tread lightly on the earth, but as our global reach has expanded so have our impacts on other species. Vanishing habitat caused by human activity is the number one threat to biodiversity, but the dispersal of alien invasive species—again, caused by humans—is not far behind. Over 4,500 non-native plant and animal species have established residence in the United States since European settlement, according to a 1993 report by the US Office of Technology and Assessment. Many alien species cause little disturbance, while others radically transfigure their new habitat by displacing less competitive native species and disrupting fragile ecological relationships that evolved over millions of years.

Of a growing list of invasive plants in North America, garlic mustard (*Alliaria petiolata*) has been on the Nature

Conservancy’s Red Alert list since 2000. Originally found in Europe, it was planted in the late 1860s by European settlers for its medicinal and culinary properties. The weed has since spread from New York to Canada and 30 US states in the East and Midwest, with recent sightings as far west as Oregon. Many mechanisms have been proposed to explain the success of alien plant invasions, mostly related to the absence of natural predators or parasites or the disruption of long-established interactions among native organisms. Few studies, however, have directly tested these possibilities. In a new study, Kristina A. Stinson, John N. Klironomos, and colleagues do just that by investigating garlic mustard’s effects on native hardwood North American trees. The weed gains a competitive advantage, they discovered, by releasing



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Severe invasion by garlic mustard in a maple-dominated forest understory in New England. (Photo: Benjamin Wolfe)

chemicals that harm a fungus the trees depend on for growth and survival.

Many forest trees and other vascular plants form mutually beneficial relationships with arbuscular mycorrhizal fungi (AMF). The fungus has long filaments that penetrate the roots of plants (forming branched structures called arbuscules) and snake through the soil in an intricate interwoven network of mycelium, which effectively extends the plant's root system. AMF depend on the plant for energy, and the plant depends on the fungus for nutrients. Many non-native plants, including garlic mustard, do not depend on native AMF and often take root in landscapes altered by development or logging, where AMF networks are disturbed. When these non-mycotrophic invasives propagate, they may diminish AMF densities even further.

Biologists are especially concerned about what might happen if a non-mycorrhizal invasive plant turns up in a mature, intact forest with an established mycelial network—which is just what garlic mustard has started to do. In the North American forests it has recently invaded, the plant inhibits the growth of understory plants, including the seedlings of canopy trees. Stinson et al. suspected the invader might somehow be thwarting the symbiotic relationship between fungus and tree.

To test this possibility, they collected soil from five forests in Ontario dominated by four species of native hardwoods. Soil was taken from infested and uncontaminated areas from each location. First, the researchers tested seedlings'

ability to form mycorrhizal relationships in soil with a history of garlic mustard invasion. Three species—sugar maple, red maple, and white ash—had significantly less AMF root colonization and slower growth when grown in the infested soil. Seedlings grown in sterilized soil taken from invaded and pest-free locations showed similar reductions, suggesting that diminished microbial activity led to suppressed growth.

A second set of experiments supported this conclusion by showing that native trees grown in soils conditioned with garlic mustard (weeds were grown in soil, then removed) had lower AMF colonization and impaired growth than when grown in soil conditioned by native plants. Since adding extracts of garlic mustard impaired AMF colonization and seedling growth as effectively as the whole plants did, the researchers concluded that garlic mustard uses phytochemical poisons to disrupt native plants' mycorrhizal associations and stunt their growth.

Stinson et al. go on to show that garlic mustard's impacts vary with a native plant's AMF dependency. Plants with fewer roots to take up nutrients—like the hardwood seedlings studied here—will be most affected by garlic mustard invasions. This suggests that garlic mustard is invading the understory of mature forests because it's poisoning the lifeblood of its woody competitors. If true, the appearance of this noxious weed in an intact forest promises to have devastating impacts. First the plant will stifle the regeneration of the dominant canopy trees, and then it will pave the way for weedy plants that don't like the beneficial fungi.

Which phytochemicals are to blame and how they interact with other beneficial soil microbes is a question for future study. Determining if and how plants in garlic mustard's native European habitat peacefully coexist may suggest ways to help North American natives fend off its fungicidal attacks. With evidence that the plant can displace native species within ten years of establishing a presence, prudence suggests taking steps to eradicate the weed before all the answers are in.

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, et al. (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. DOI: 10.1371/journal.pbio.0040140

A Neural Seat for Math?

Liza Gross | DOI: 10.1371/journal.pbio.0040149

Abstract mathematical reasoning is often treated as a uniquely human endeavor. But many species, from pigeons to primates, show some ability to grasp the concept of number, suggesting that these numerical abilities represent the evolutionary building blocks of higher math in humans. Sophisticated symbolic number processing in adults recruits a region of the brain called the intraparietal sulcus (IPS). While children can grasp basic math concepts relating to size and number before they know the words that describe them, very little is known about the neural basis of these abilities. Does the IPS support the relatively simple numerical tasks of childhood as well as the sophisticated numerical calculations that adults learn to perform?

Behavioral studies show that adults respond similarly to nonsymbolic numerical stimuli (arrays of dots) and symbolic



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The brain circuits for comprehending math are already in place early in development.

numerical stimuli (Arabic numerals), suggesting that a common pathway supports both tasks. But neuroimaging studies have not resolved whether the same brain pathway is involved in both symbolic and nonsymbolic number processing. In a new study, Jessica Cantlon, Elizabeth Brannon, Elizabeth Carter, and Kevin Pelphrey at Duke University used functional magnetic resonance imaging (fMRI) to investigate how the IPS responds to nonsymbolic numerical values in adults and preschool-aged children. They show that the brain circuitry governing nonsymbolic number processing is already in place very early in human development.

In the study, adults and four-year-olds lay in a scanner while passively viewing a continuous stream of visual arrays on a computer screen. The arrays were designed to elicit differences in brain response to stimuli that were either novel in number or novel in shape. This study design operates under the assumption that neurons tuned to a particular stimuli (numbers, for example) will stop responding when exposed to a standard stimulus (16 circles) over and over, but will respond to stimuli that deviate from the norm (six or 32 circles). Every so often, a deviant number or shape (a triangle or square in place of a circle) was mixed in with the standard stimuli. Participants pressed a button when a crossbar in the center of the visual

display turned red to maintain focus. Cantlon et al. analyzed the fMRI data to determine which brain regions responded to both types of deviant stimuli in the adults and children.

Number deviants produced a much greater bilateral response in the IPS of adults compared with shape deviants, with activity extending into the inferior and superior parietal lobules (SPL). This response was confirmed by an alternate measure of brain activation based on blood oxygen level, which rose significantly three to 7.5 seconds after the number of elements changed. Brain regions that responded to shape deviants were concentrated in the ventral temporal-occipital cortex.

fMRI results for the children showed that number deviants produced a significant response in and around the right IPS and the right SPL. Brain response to shape deviants was similar to that observed in adults. The location and pattern of brain activity in the preschoolers resembled that reported in studies of nonsymbolic numerical processing and basic math ability in adults. By four years old, children's brains already selectively respond to nonsymbolic numerical values, suggesting that the neural networks for number processing are established early in life.

How to explain the finding that IPS activity was bilateral in adults and concentrated in the right hemisphere in the four-year-olds? It could be that the left hemisphere acquires more sophisticated math-related functions over time while the right remains relatively stable. But since some children showed more activity in the left IPS, the researchers warn that future study will have to determine whether this pattern is unique to kids.

Overall, these results indicate that the brain dedicates a region to cultivating numerical abilities early in development. The IPS provides the neurobiological platform for nonsymbolic numerical processing in young children, then supports the expanding capacity for higher-math operations in adulthood. Six-month-olds also have an abstract numerical sense, suggesting that the IPS may even underlie numerical processing in infancy. Much remains to be learned about how children learn to count and match words with symbolic representations of numbers, but these results suggest that focusing on the IPS might help relate biology to behavior to answer some of these questions, and perhaps shed light on the evolution of numerical cognition.

Cantlon JF, Brannon EM, Carter EJ, Pelphrey KA (2006) Functional imaging of numerical processing in adults and 4-y-old children. DOI: 10.1371/journal.pbio.0040125

For Arthropod Mitochondria, Variety in the Genetic Code Is Standard

Richard Robinson | DOI: 10.1371/journal.pbio.0040175

The protein-making instructions of DNA, and the RNA messages transcribed from them, are spelled out in nucleotides. Proteins, though, are written in amino acids, and one of the seminal discoveries of the early days of molecular biology was the code that relates one to the other. Each of the 20 amino acids is represented by one or more unique RNA triplets, or codons: UAC is decoded as tyrosine, for example, and UGC as cysteine. (U is

the RNA nucleotide containing uracil, A is adenine, C is cytosine, and G is guanine.)

For a decade or so after its discovery, the code was believed to be universal, exactly the same in every organism, from bacteria to bonobos. But exceptions—variations in the coding of one or two amino acids—soon turned up, particularly in mitochondria, the subcellular powerhouses in all our cells that have descended from once

free-living bacteria. (Mitochondria contain their own DNA and protein-producing machinery, and reproduce independently from the host cell.) Indeed, most of the nonstandard codes discovered to date have been found in the mitochondria of different animal lineages. While there are differences between some animal phyla (chordates, mollusks, and echinoderms, for example), nonstandard mitochondrial codes within an animal phylum have all

been considered the same, which has been interpreted to mean that these nonstandard codes arose very early in each lineage and remained unchanged thereafter.

In a new study, Federico Abascal, Rafael Zardoya, and colleagues develop a new analytic technique to show that within one animal phylum—the arthropods—there are two nonstandard codes, and suggest that genetic code changes within a lineage may be more frequent than was earlier believed.

To identify nonstandard mitochondrial genetic codes, the authors compared the mitochondrial coding sequences from 626 different animal species, aligning the sequences to find codons conserved within a gene from one species to the next. They then asked what amino acid any particular codon specified in the protein. The most frequent AA was taken to be the canonical translation of that codon. From there, they could ask whether that same codon is translated as that amino acid in any particular species, in this way identifying potential variant genetic codes. Not every codon position in every gene is conserved between species, of course, and the art of this procedure lies in finding a balance between stringency and tolerance in aligning codons from imperfectly matched sequences. Rigorous exclusion of all misaligned positions produces few but certain data, while a more tolerant approach to



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The horseshoe crab uses the newly discovered genetic code (AGG translates into lysine rather than serine). Parallel evolution of this and the typical code of invertebrate mitochondrial genomes (which is correlated with tRNA mutations) occurred repeatedly along the evolutionary history of arthropods. (Figure: horseshoe crab lithograph by George Endicott)

mismatches produces more but noisier data. By varying stringency and testing the results against a small set of well-characterized genomes, they arrived at a robust computational approach to analyzing new mitochondrial genomes for nonstandard codons.

They found that while almost every codon translated into the expected amino acid (as deduced from the annotated genetic code) in all species, there was a surprising trend in the arthropods, the largest of all animal phyla, which includes the insects,

crustaceans, spiders, and other similar creatures. Among mitochondria from all invertebrates, AGG typically translates as the amino acid serine. Among the 92 mitochondrial genomes from the arthropods, however, AGG coded for serine in 34 species and lysine in 24 other species. Among the rest, the meaning could not be deduced in 18, and 34 species did not use the AGG codon. The authors' analysis of the patterns of change also suggests that the original arthropod mitochondrion used AGG for lysine, not serine.

The sequence of reassignment, disuse, and reversion to the original is difficult to tease out for any lineage within the arthropods, but the variety within the group suggests the code has changed multiple times between the two genetic codes. One explanation for this variety is that pairing of AGG and lysine is disadvantageous for the organism employing it, so that loss or reversion over time would be favored. If true, this explanation suggests there may be multiple other nonstandard codes residing within other lineages that began with a nonstandard and selectively unfavorable coding change. Further application of the authors' analytic method may decode more such surprises in the future.

Abascal F, Posada D, Knight RD, Zardoya R (2006) Parallel evolution of the genetic code in arthropod mitochondrial genomes. DOI: 10.1371/journal.pbio.0040175

Relaxing the Clock Brings Time Back into Phylogenetics

Richard Robinson | DOI: 10.1371/journal.pbio.0040106

The hypothesis of a “molecular clock”—a constant rate of mutation over evolutionary time—revolutionized phylogenetics, the study of evolutionary relationships among organisms. Using the assumption of this constant rate, one can determine the time since two organisms diverged from a common ancestor simply by toting up the number of DNA sequence differences between them. Thus, the molecular clock provided an important tool for constructing phylogenies, “trees” of relatedness, for organisms as diverse as primates and protists.

However, the constancy of the mutation rate, both between different groups and within a single group over time, has been repeatedly challenged. As a result, the molecular clock has been largely abandoned in recent years for constructing phylogenetic trees. In its place has arisen a model that accepts that each branch may have its own rate of mutation. Relatedness between two organisms can still be determined and trees can still be drawn, but without a constant mutation

rate, no estimate can be made of the time since divergence, and thus the position in time of the last common ancestor—the “root” of the tree—cannot be calculated.

An alternative approach, termed a “relaxed molecular clock,” has been developed to overcome the difficulties of both the molecular clock and unrooted phylogeny models. In a new study, Alexei Drummond, Andrew Rambaut, and colleagues describe a new approach to relaxed-clock analysis, showing that it can be used to simultaneously construct accurate trees and infer times of divergence.

Previous attempts to reintroduce a molecular clock into relaxed phylogenetics have posited differing, but correlated, rates of mutation along different branches. But filling in these rates requires specifying the topology of the tree—knowing who's related to whom—beforehand. This is often poorly known, and may be the very question phylogeneticists are trying to answer.

Drummond et al. took a different approach. Using a set of artificial DNA sequences generated and mutated to form a rooted tree, they tested five different models of rate variations to determine which most accurately modeled the simulated evolution of this group. The five models included a strict molecular clock, in which mutation rates were the same on all branches at all times, as well as various modifications in which rates were correlated or uncorrelated among the branches. Using the phylogenetic analysis program called BEAST, they found that the most robust model—the one that did best under various starting conditions—was neither the strict molecular clock nor the correlated models, but the “uncorrelated relaxed-clock” models, in which the mutation rates in each branch are allowed to vary but within particular constraints.

They then tested their models in several real sets of data, including viruses, marsupials, plants, bacteria, and yeast. In

the plant dataset, which was known to have the most “clock-like” evolution, the strict molecular clock model did best, not surprisingly. But the relaxed-clock model performed best overall among all the datasets, drawing trees that were closest to known relationships with the fewest missteps. And, unlike in the unrooted phylogenetic approach, they were able to assign times of divergence to each branch on the tree.

The model developed by the authors promises to bring the very important question of time back into phylogenetic analysis. The ability of the model to create accurate trees may also make it of use even to scientists whose main interests are in understanding phylogenetic relationships, rather than the timing of evolutionary divergence.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. DOI: 10.1371/journal.pbio.0040088

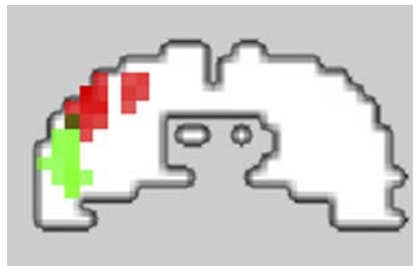
Functional Connections in the Brain Transform Experience into Memory

Liza Gross | DOI: 10.1371/journal.pbio.0040165

The ephemeral quality of memory is captured in a classic Steven Wright joke. “The other day I . . . no, wait,” he deadpans. “That wasn’t me.” Most real-life bouts of episodic memory loss are more mundane—what did I have for dinner last night?—and perfectly normal. And considering the constant stream of stimuli most people encounter in a single day, it’s a wonder anyone remembers anything.

To process new experiences, discrete regions in your brain transform a rich palette of perceptual information into an internal representation of the experience. Over time, different regions of the brain consolidate the neural traces of these episodes into more enduring memories. Behavioral and neuroimaging studies have implicated a network of brain structures—including the medial temporal lobes (MTL), prefrontal cortex (PFC), and sensory cortical areas—in episodic memory formation. How these memory centers collaborate, and particularly what role the PFC plays, remains a subject of debate.

Neuroimaging techniques are typically used to link brain activity with a particular task or function. In a new study, Christopher Summerfield, Jennifer Mangels, and colleagues take functional magnetic resonance imaging (fMRI) a step further to determine the functional connections across multiple brain regions during episodic memory processing. They show that functional links between the dorsolateral PFC and



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Regions of the dorsolateral prefrontal cortex found to exhibit significant connectivity with face-sensitive (red) and place-sensitive (green) visual regions during the formation of new associations between faces and places.

two regions of the sensory neocortex predict how well people remember associations between images of faces and houses.

They propose a model whereby the PFC exerts “top down” control over episodic memory processing by modulating activity in the sensory cortex to select which inputs go into a new memory trace. These inputs are selectively channeled through different stages of the processing hierarchy until they reach MTL structures, such as the hippocampus, for consolidation into long-term memory. Previous neuroimaging studies have shown that blood flow changes in areas of the PFC and sensory cortex vary as a function of what neuroscientists call “encoding success”—the likelihood of forming a new long-term memory. And activation of brain regions associated

with learning new associations can often predict a participant’s encoding success. But stronger support for this model, Summerfield et al. argue, would come from showing that functional connectivity between different brain regions in the processing hierarchy can also predict encoding success.

To look for evidence of functional connectivity, the researchers focused on two regions of the extrastriate visual cortex that selectively respond to faces—the fusiform face area (FFA)—and houses—the parahippocampal place area (PPA). In the first phase of each of 20 blocks (groups of stimuli), participants viewed seven consecutive pairs of faces and houses and were asked to memorize (or “intentionally encode”) associations between faces and houses. During the testing phase, participants viewed the original pairs, interspersed with seven new pairs, remixed from the original images. Participants indicated whether the pairs were old or new by pressing one of five buttons, indicating confidence in their choice. The authors predicted that FFA and PPA responses would track memory performance (encoding success), and that connectivity between the FFA/PPA regions and the PFC could predict performance.

A search for brain regions in which responses related to learning the associations differed from subsequent memory of the image pairs identified a region associated with early visual processing, the lateral occipital

complex (LOC), as well as the FFA, PPA, and MTL. Later memory effects were also seen in the vascular response, with peak responses occurring progressively later in the processing hierarchy—a pattern that might reflect the ongoing processing of task-relevant perceptual codes that go on to the next stage of processing.

To determine functional connectivity, the researchers focused on state-related responses. State-related activity—rather than stimulus-evoked responses—they explain, may reflect ongoing brain activity that is

not limited to a stimulus but helps regulate its integration into memory formation. By correlating encoding success with connectivity between the left dorsolateral PFC and the FFA/PPA, they showed that connectivity predicted later memory performance for each participant. They go on to show that connectivity between this PFC region was “reliably greater” than connectivity between the PFC and other regions in the processing hierarchy.

By showing that connectivity can predict behavioral performance, these results provide support for the

top-down model in which the PFC regulates activity in lower cortical regions to cherry pick representations most relevant to the task at hand. This model may shed light on other modes of neurocognitive processing as well. The next big challenge will be to figure how individual neurons mediate these functional connections across multiple brain regions.

Summerfield C, Greene M, Wager T, Egner T, Hirsch J, et al. (2006) Neocortical connectivity during episodic memory formation. DOI: 10.1371/journal.pbio.0040128

Global Analysis of a Key Developmental Pathway in Plants

Liza Gross | DOI: 10.1371/journal.pbio.0040172

Stem cells are most often associated with mammalian development, but plants have them too. With a modular architecture that allows ongoing replacement of new stems, leaves, roots, and flowers, plants escape the debilitating effects of aging that other multicellular organisms endure. The raw materials for regeneration come from reserves of stem cells—sequestered in two meristems, one for shoots and another for roots—that adult plants draw on throughout their lives.

The root meristem consists of the quiescent center (QC)—a group of four cells that maintain neighboring cells as stem cells—and the undifferentiated “initial cells” that give rise to the concentrically organized cylindrical root tissues: the epidermis, ground tissue (made up of the cortex and endodermis), and stele (pericycle and vascular cylinder). Initial cell divisions are asymmetrical, resulting in one renewed initial cell and a daughter cell that differentiates. A key regulator of root development, the transcription factor SHORT-ROOT (SHR) acts in the QC and endodermis to regulate stem-cell specification and radial patterning.

The genetic workhorse of plant biology *Arabidopsis thaliana* has shed considerable light on the molecular pathways controlling these processes, yet many details remain obscure. In a new study, Mitch Levesque, Teva Vernoux, Philip Benfey, and colleagues focused on SHR to better understand its role in root development and identify those genes that are directly controlled by it. Before their study, only one of SHR's gene targets, *SCARECROW (SCR)*, had been identified. The researchers also demonstrate the value of applying meta-analysis—a standard statistical approach used in many other fields to integrate and interpret the results of multiple independent studies—to the analysis of transcriptional networks in development.

To identify the targets of a transcription factor, researchers typically alter their activity and then analyze genome-wide transcription with microarray analysis—an approach that proves cumbersome in multicellular organisms, where genes are often expressed in different cells at different times. Meta-analysis can overcome this problem, Levesque et al. argue, because it can detect subtle patterns in larger datasets that might be overlooked in smaller ones. Using this approach, the researchers identified eight direct SHR transcriptional targets, including *SCR*, as well as a long list of indirect targets



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Arabidopsis thaliana.

involved in cell signaling and hormonal responses. They also revealed a new function for the transcription factor.

First, they constructed a form of SHR that allowed them to exert precise control over its temporal and spatial expression by administering a synthetic hormone called dexamethasone (Dex). Plants lacking *SHR* (*shr-2* mutants) have much shorter roots and defective radial patterning. But *shr-2* mutants bred to express the SHR construct had normal root growth, which the researchers attribute to restored stem-cell activity. By crossing this strain with another engineered to express a fluorescent protein upon *SCR* transcription, the researchers could predict when SHR targets were expressed after Dex treatment. Then, adding a compound (called cycloheximide) expected to block expression of genes that act further downstream in a pathway, they demonstrated that SHR directly targets *SCR*.

To identify other direct SHR targets, the researchers altered SHR activity in their transgenic *shr-2:SHR* mutants using three different experimental treatments, then collected transcriptional profiles from the root tips of five-day-old plants. The meta-analysis of the three microarray datasets identified eight candidate targets—four of which can bind to

SHR in plant cells. Which is not to say that SHR can't bind to the other four genes, only that these assays did not provide that evidence. Future studies can resolve this question.

All eight candidate targets were expressed to some degree in cell types expressing SHR. Three target genes were expressed in stele tissues, the root's vascular tissue. Levesque et al. found that the stele was narrower and had fewer initial cells in *shr-2* mutants, demonstrating a new role for SHR in stele differentiation and development.

The researchers go on to show that hundreds of indirect target genes are either activated or repressed in response to SHR activity and that many of these genes are involved in cell or hormonal signaling pathways. They plan to investigate the functional significance of these genes in future experiments.

Overall, these results suggest that SHR directly activates *SCR*, and in doing so influences QC specification and

asymmetric cell division. It does not work alone, however, since simply adding *SCR* to *shr-2* mutants did not correct defects associated with these processes. SHR operates in at least five regions, Levesque et al. conclude: the QC, early and late endodermis, and early and late stele. SHR controls root development, they propose, by coordinating overlapping transcription, signaling, and hormonal pathways. The product of these interactions determines how SHR influences stem-cell niche specification, radial patterning, and stele development. Functional analysis of the different targets will help the researchers test the validity of their model.

Levesque MP, Vernoux T, Busch W, Cui H, Wang JY, et al. (2006) Whole-genome analysis of the short-root developmental pathway in *Arabidopsis*. DOI: 10.1371/journal.pbio.0040143

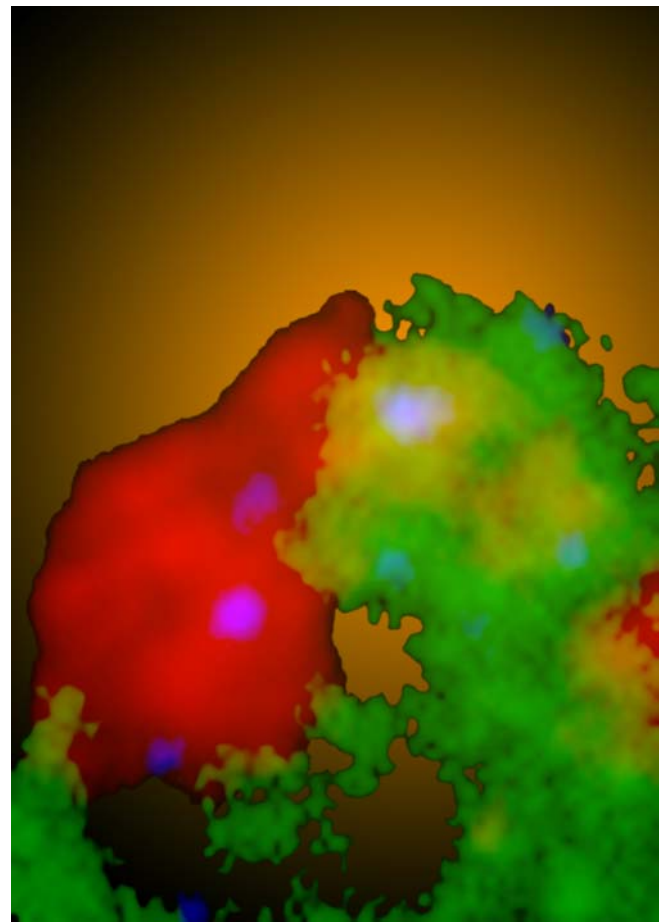
Interphase Chromosomes Mingle with Their Peers

Françoise Chanut | DOI: 10.1371/journal.pbio.0040174

Though chromosomes appear as discrete, tidy rod-like bodies with distinct sizes and shapes during cell division, they unravel and morph into what looks like a tangled ball of yarn at the end of each division, when they re-form the cell's nucleus. Nevertheless, experimental evidence from the past 20 years suggests that they remain separate entities throughout the cell cycle. A new study by Miguel R. Branco and Ana Pombo now calls this evidence into question by showing that chromosomal interactions are frequent in the nucleus of human cells during interphase, the part of the cell cycle that lies between cell divisions.

The yarn that fills the interphase nucleus is chromatin, which consists of DNA coiled around histone proteins. In the 1980s, cell biologists developed a technique they termed FISH (for fluorescence in situ hybridization) that allowed them to stain each chromosome a different color. FISH staining showed that even in their unfolded state, chromosomes allow very little—if any—mingling of their chromatin. But FISH requires a harsh chemical treatment that is known to alter chromatin structure. Branco and Pombo developed a modified FISH technique that maintains chromatin integrity and improves the resolution of chromosome visualization. Using this technique, they uncovered more intermingling among interphase chromosomes of human cells than previously observed. Further experiments suggest that intermingling plays an important part in chromosome structure and gene expression.

FISH is normally performed on intact nuclei, to preserve the three-dimensional arrangement of chromosomes. By contrast, Branco and Pombo carried out their staining on cells they had previously frozen and sliced into ultrathin sections. The dyes were able to penetrate the thin samples easily, which eliminated the need for aggressive detergents that would disrupt chromatin organization. The researchers applied various pair-wise combinations of dyes to their ultrathin sections and scored as intermingling any spot of overlapping dye signals. Models based on classic FISH experiments suggest that chromosome territories (CTs), each of which contains the chromatin of a single chromosome, are separated by a protein matrix called an interchromatin domain (ICD.) But Branco and Pombo found that each chromosome mingles on



DOI: 10.1371/journal.pbio.0040174.g001

Two different human chromosomes “painted” in green and red are shown to intermingle in the yellow area.

average 2 percent of its chromatin with the chromatin of any other chromosome. Given that human cells contain 23 pairs of chromosomes, this means that intermingling might affect 46 percent of the volume of any chromosome, which poses a severe challenge to the notion of a distinct ICD compartment.

When they examined areas of intermingling at the higher resolution afforded by electron microscopy, Branco and Pombo found that DNA sequences from two mingling chromosomes came into close enough proximity to interact at the molecular level. This observation prompted them to wonder whether intermingling is related to biological functions such as DNA repair and gene expression, both of which rely on the bridging of distant pieces of DNA by large protein complexes.

DNA repair mechanisms are activated when chromosomes break, for instance after prolonged exposure to radiation. When the repair occurs by re-joining the broken ends of two distinct chromosomes, a translocation ensues. In irradiated lymphocytes, translocations occur with various frequencies between various chromosome pairs. Branco and Pombo found a strong correlation between the extent of intermingling and the frequency of translocation for given

chromosome pairs. They conclude that intermingling areas are privileged sites for the occurrence of translocations.

To demonstrate a link between chromosome intermingling and gene expression, the researchers inhibited the major lymphocyte's RNA polymerase, one of the enzymes that transcribes DNA sequences into RNAs. Intermingling decreased for some chromosomes and increased for others, confirming that intermingling patterns are molded by a cell's transcriptional activity. Because different cell types express different subsets of genes, intermingling may explain why different cell types are prone to different chromosomal rearrangements. For more on visualizing chromosomal territories, see the related Primer (DOI: 10.1371/journal.pbio.0040155).

Branco MR, Pombo A (2006) Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. DOI: 10.1371/journal.pbio.0040138

A New Test Detects Selection for Early Flowering in a Much-Studied Weed

Liza Gross | DOI: 10.1371/journal.pbio.0040171

Plants use a wide range of reproductive strategies to get around the fact that they can't pull up stakes when conditions deteriorate. The life cycle of flowering plants precisely tracks local daylight and temperature cycles to optimize flowering and boost reproductive success. The timing of flowering is crucial. As any temperate-gardener knows, a delicate plant that flowers too early in the season is doomed to perish with the next frost.

Much has been learned about the genes plants use to synchronize flowering with favorable environmental conditions by studying *Arabidopsis thaliana*. This slight mustard weed grows throughout the Northern Hemisphere. Reflecting adaptations to local environments, plants from different locations flower at different times when grown under the same light and temperature conditions. In particular, many plants take a very long time to flower unless induced to do so by prolonged exposure to cold in a process known as vernalization (the same process that forces spring bulbs into early bloom). This requirement for vernalization has been linked to the *FRIGIDA (FRI)* gene, based on observations that plants with nonfunctional *FRI* variants, or alleles, flower early without forcing. Two of these loss-of-function alleles—*fri_{Col}* and *fri_{Ler}*—are linked to many of the early-flowering plants found in Europe.

There's a good chance that a gene associated with a trait directly related to reproductive success would show signs of selective pressure—and that's just what a new study shows. Christopher Toomajian, Magnus Nordborg, and their colleagues developed a novel genomics-based approach to detect selection and provide evidence that the two *FRI* alleles are under selection for rapid flowering in *Arabidopsis*.

A standard approach for detecting selection on a particular genomic region relies on the theoretical predictions of the neutral theory of molecular evolution. The problem with this approach, Toomajian et al. argue, is that many other forces besides selection can cause a deviation in the data from what is expected under simple neutral models. And with the plethora of genomic polymorphism data, they explain, it's possible to forgo the models and compare genome-wide patterns of variation instead. If the pattern at a region of



DOI: 10.1371/journal.pbio.0040171.g001

Gene variants that allow *Arabidopsis* plants to flower early in the season without "forcing" turn this annual weed into a rapidly reproducing superweed.

interest differs radically from the genomic pattern, the region may be under selection.

A 2002 study of polymorphism around the *FRI* locus found that plants carrying one of the early flowering alleles also shared long blocks of identical chromosomal regions, or haplotypes. Building on those results, the researchers looked for patterns of haplotype sharing in genomic data from 96 *Arabidopsis* plants to see if the length of these haplotypes was typical, in which case the region probably wasn't under selection, or unusual, in which case it probably was.

To compare haplotype sharing around the *FRI* alleles with sharing at thousands of other loci, the researchers developed a new test, called the pairwise haplotype sharing (PHS) score. This score includes a function that controls for population structure: since pairs of individuals from the same population are more closely related than those from different populations, they're more likely to share long haplotypes and could bias the results.

PHS scores were calculated for all alleles found in the dataset, including the two *FRI* alleles, which had abnormally

high haplotype sharing scores compared with the “vast majority” of alleles. Thirty-one other alleles also showed higher-than-average PHS scores, which the researchers plan to investigate as possible selection candidates in a future study. They also measured haplotype sharing using another test developed by Pardis Sabeti and colleagues, called extended haplotype homozygosity, which relies on the relationship between an allele’s population frequency and its linkage to surrounding loci to determine its likelihood of being under selection given its age. This measure, they show, identifies multiple alleles from very similar sets of individuals that tend to come from the same population. This property can erroneously attribute haplotype sharing caused by population structure to selection. But this potential drawback shouldn’t be a problem for organisms with a less complex population structure, like humans.

The researchers acknowledge that scanning the genome for haplotype sharing can’t pinpoint the target of selection,

but these results strongly suggest an adaptive role for the *FRI* alleles. While the evidence for selection is stronger for *fri^{Col}* than for *fri^{L_{er}}*, it may be that selection on *fri^{L_{er}}* happened much earlier, since both alleles have similar effects on the plant. This possibility is supported by the estimated age of the alleles, which the researchers place at 800 generations ago for *fri^{Col}* and 3,200 generations ago for *fri^{L_{er}}*. Thus, if one assumes at least one generation per year, the loss-of-function *FRI* alleles emerged on the heels of the last glacial retreat, some 13,000 years ago. The researchers speculate that the evolution of these early flowering alleles, which turn an annual weed into a rapid reproducer, reflects selection for “weediness” in response to agriculture—an intriguing possibility that future studies can explore.

Toomajian C, Hu TT, Aranzana MJ, Lister C, Tang C, et al. (2006) A nonparametric test reveals selection for rapid flowering in the *Arabidopsis* genome. DOI: 10.1371/journal.pbio.0040137

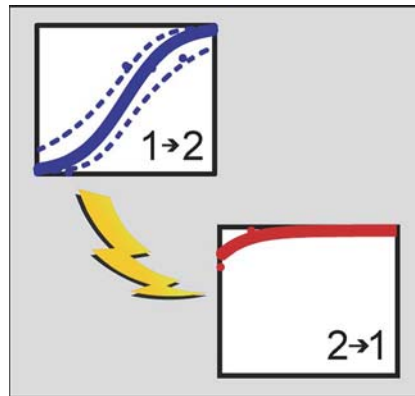
Evidence for the Encoding of a Motion’s Goal in the Monkey Brain

Françoise Chanut | DOI: 10.1371/journal.pbio.0040169

From cooking to playing the piano, our activities consist of simple motions that we string together for specific purposes. When we learn a new recipe or piano sonata, we memorize the elemental gestures of the trade as well as the precise order in which they must occur to produce a *coq au vin* or “Moonlight Sonata.” And when we start cooking or playing, we somehow summon the sequence of movements that will lead to our goal.

Scientists have long wondered how the brain stores in memory a sequence of movements. Ultimately, such memories result from connections that brain cells (neurons) establish among themselves and with the relevant muscles during the learning period. But precisely how the information is organized in the brain remains largely mysterious. A model called “associative chaining” proposes that a memory cell representing an early motion activates memory cells representing later motions in the sequence. In this model, a memory cell recalls at once the type of motion and its order of appearance in the sequence. But in a new study, Mark H. Histed and Earl K. Miller show that they can uncouple the memories for a sequence’s components and for their serial order by manipulating a subset of cells in the brain of monkeys. Their observations suggest the existence of abstract forms of memory that store the goal of a sequence of movements.

The researchers focused on a small area near the surface of the brain’s



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The sequential order of short-term memory can be reversed by using tiny amounts of electrical current to change neural activity in the frontal lobe of the brain.

frontal lobe called the supplementary eye field (SEF). Within the SEF are cells that control small voluntary eye movements called saccades that animals use to track bright objects near the periphery of their field of vision. Stimulation of individual SEF neurons triggers saccades to separate locations, and SEF neurons have been found to fire sequentially while monkeys made serial saccades to visual targets, as if the cells controlled the saccade succession. The SEF therefore appeared like a logical place to look for neurons that harbor memories of learned saccade sequences.

Histed and Miller first trained two monkeys to saccade to successive locations on a screen. While the

monkeys focused on a bright central spot, two additional spots (cues) would appear at random one after the other and disappear after half a second. The monkeys had to wait one more second before saccading to the two cued locations in the order of the cues’ appearance. The task therefore required memorizing both the cues’ location and order. The monkeys were rewarded each time they accomplished a saccade sequence correctly, and they eventually performed with a near-perfect score. In an experimental session, the researchers would stimulate various sites in the SEF with microelectrodes during the one-second delay period, and record the effect of these stimulations on the monkeys’ performance.

Out of 55 SEF locations tested, the researchers found 25 that disrupted the monkeys’ performance. The pattern of errors was always the same: the monkeys saccaded to the correct locations, but in incorrect order. For instance, if the sequence consisted of a cue at a 1 o’clock location followed by a cue at 3 o’clock, the monkeys would saccade to 3 o’clock first and then 1 o’clock. However, they never saccaded to 5 o’clock or 11 o’clock, or to any other wrong location. Therefore, the monkeys seem able to keep the memory of visual cues intact even when they forget the order of the cues’ appearance, which shows that memories for the order versus

the components of a sequence are held separately in the monkey's brain. More specifically, these results indicate that the SEF can reorganize a series of saccades.

Strong stimulation of the SEF can elicit eye movements. If you vertically divide the visual world in half, stimulating the right SEF causes saccades to the left field, and vice versa. But the stimuli Histed and Miller applied to the SEF were

too weak to produce saccades. Nevertheless, their effects were similarly biased: they reordered saccade sequences such that the monkeys always seemed to aim for left field when receiving a stimulus in the right SEF, and to right field when receiving a left stimulus. The researchers conclude that rather than precisely ordering a sequence of saccades, the SEF codes for a spatial goal (left field or right field),

regardless of whether it takes one or more saccades to reach that goal.

The implication of these results is that the brain might store complex sequences of movements in two forms: one for the sequence's goal and one for the details of its execution.

Histed MH, Miller EK (2006) Microstimulation of frontal cortex can reorder a remembered spatial sequence. DOI: 10.1371/journal.pbio.0040134

Bats Keep Their Ear on the Ball

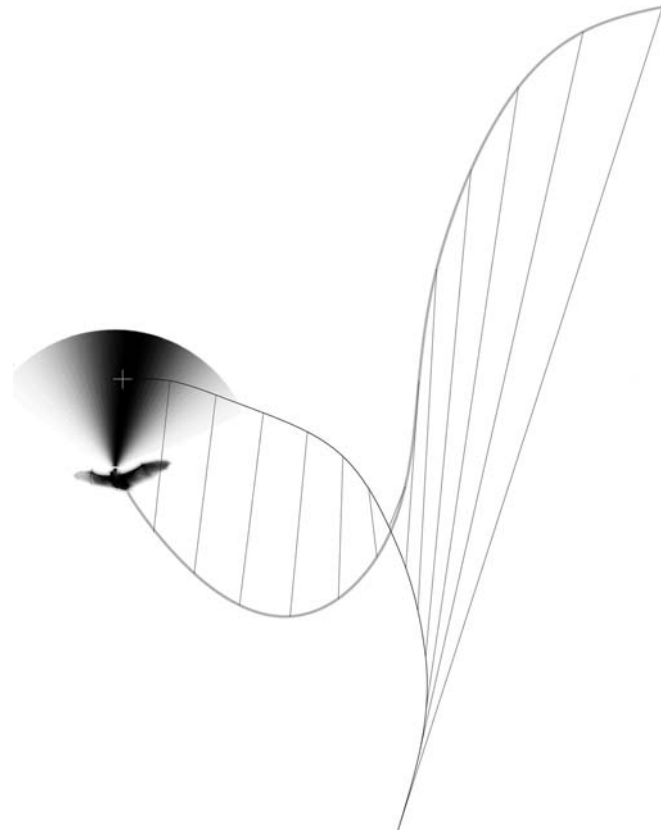
Françoise Chanut | DOI: 10.1371/journal.pbio.0040152

Imagine you are late for your train. As you approach within sight of the station, the last car pulls off to the left. So you start running in a diagonal to catch up with it. The difficulty is deciding how much to bear left. A different angle will be needed depending on both your speed and the train's speed. For a given set of speeds, mathematics dictates that there is only one bearing angle that will put you on that train.

Determining that optimal bearing and staying the course are the essence of constant bearing (CB), a strategy that sailors, ballplayers, and animals on the prowl use to intercept (or avoid) moving targets. CB is the optimal strategy as long as the target follows a predictable course. Using mental projections of the target's and their own trajectories, a fish pursuing sinking bait and an outfielder tracking a fly ball compute their optimal bearing and move in a straight line toward the point of intercept, adjusting their course along the way if necessary. With targets that change speed and direction unpredictably, a pursuer may still use CB successfully over the stable segments of the trajectory, which is how dogs catch swerving Frisbees. But erratic targets, which change speed and direction quickly and randomly, may not leave the pursuer enough time for CB computations.

Zeroing in on erratic targets is a matter of survival for the big brown bat, which must snag fitfully flying insects before they return to the safety of foliage. Still, a second or two is all a bat needs to locate and catch a straying beetle on the wing, suggesting bats have an efficient way of tracking their prey's erratic motions. Kaushik Ghose, Cynthia Moss, and colleagues observed big brown bats capturing flying insects in a laboratory setting. They report in a new study that, rather than CB, the bats rely on constant absolute target direction (CATD), a strategy that can be shown mathematically to be most time efficient for tracking erratic motions and is similar to that used by search missiles to latch onto moving targets.

Big brown bats use sound rather than light to sense their surroundings. They emit short ultrasonic shrieks into the air and listen to the returning echoes to detect objects around them, quickly adjusting their flight to approach an insect or avoid an obstacle. The researchers released a big brown bat and a flying insect in a large dark room. Using high-speed infrared cameras and a battery of microphones, they recorded the flight path of bats and insects, as well as the bats' shrieks and echoes. Computer analyses of the recordings allowed them to measure the speed and direction of the flights, and calculate a theoretical optimal bearing for each



DOI: 10.1371/journal.pbio.0040152.g001

An echolocating bat pursues a fast and erratically moving insect (white cross, black line) by keeping the absolute direction to the target a constant (bearing lines are parallel) while maintaining a lock on the target with its sonar beam (grayscale fan pattern).

point on the bat's path. From the direction of the shrieks and echoes, they could deduce the orientation of the bat's head relative to the insect, a situation equivalent to detecting the gaze of visual animals.

They found that after spending some time locating the insect, the bats quickly adopted the same flight path as would be predicted if they continuously recalculated bearing angles to match their prey's erratic trajectory. Rather than concluding that bats are geometry wizards, the researchers propose that they use a simple trick to cheat on the math.

One of the geometrical properties of the bat's flight path is that theoretical lines drawn from the bat to the insect would appear as a series of parallels to an external observer (hence the term CATD). Previous research has shown that the big brown bat locks its head direction to the target position—continuously looking at the target during pursuit, like a baseball player keeping his eye on the ball. The researchers suggest that when adopting the CATD strategy with its head locked to the target, all the bat has to do to simplify the math is to ensure its head does not rotate in space as its body swoops and glides to follow its prey.

In fact, the bat may possess a reflex similar to the one that links sensations of imbalance in our inner ear to a posture adjustment. This reflex would allow the bat to quickly correct any deviation of its body's alignment with the prey, thus insuring its unerring aim.

Ghose K, Horiuchi TK, Krishnaprasad PS, Moss CF (2006) Echolocating bats use a nearly time-optimal strategy to intercept prey. DOI: 10.1371/journal.pbio.0040108

Genes Affect Population Growth, but the Environment Determines How

Richard Robinson | DOI: 10.1371/journal.pbio.0040150

Why does a population in the wild grow or shrink, or remain the same over generations? Genes would seem an obvious factor, but in fact there is remarkably little evidence that genetic effects influence year-to-year population dynamics, beyond the well-recognized negative effect from inbreeding on very small populations.

This lack of evidence for a genetic effect partly reflects the difficulty of choosing which gene to study, as only a few genes



DOI: 10.1371/journal.pbio.0040150.g001

The Glanville fritillary butterfly.

may be under strong selection at any given time, and fewer still will affect population dynamics. It also partly reflects an important truth about natural selection—it may determine who survives to reproduce, but not necessarily the total number of survivors. In many cases, ecological factors such as resource abundance and natural enemies may overwhelm any slight genetic effect on population dynamics.

Finding a genetic effect, then, would require both knowing which pin in the genetic haystack to look for and having detailed knowledge of the complex and powerful environmental factors against which this effect plays out. In a new study, Ilkka Hanski and Ilik Saccheri show that variants of a sugar-metabolizing gene do indeed influence population growth in a species of butterfly, but in a complex and habitat-dependent way.

The authors studied the Glanville fritillary butterfly on the Åland Islands in Finland, where its population dynamics are well studied, and its habitat—patches of dry meadows spread across the landscape—is well mapped. They focused on the gene phosphoglucose isomerase (*Pgi*), a key enzyme in the breakdown of sugar. The *Pgi* gene occurs in several forms, or alleles, whose proteins differ in their kinetic properties and thermal stability. These alleles have been previously linked to differences in flight metabolic rate and fecundity in this butterfly, making the gene a good candidate for observing a population effect, if there is one. The *f* and *d* alleles of *Pgi* are the most common, and previous work has shown that butterflies with either an *ff* or an *fd* genotype have a higher flight metabolic rate and are more fecund than those with a *dd* genotype.

By analyzing genotypes, population growth, and habitat area simultaneously among more than 130 small butterfly populations, the authors showed that, in small meadows, growth was highest when the *ff* or *fd* genotypes predominated, but in larger meadows, the opposite was true—these genotypes predicted a decline in numbers instead of a rise, while *dd* was favored. The effect appeared to be specific to *Pgi*, as there was no correlation with genotype for any of the six other genes.

The likely explanation for this effect, according to the authors, is related to the differences in maturation and egg laying between females bearing *f* and *d* alleles. Those with *f* alleles mature quickly and lay more eggs early on, just the strategy for exploiting a small patch, from which many butterflies risk drifting away rather quickly in their life. Those with *d* alleles mature later but also die later, allowing them to exploit a larger habitat more thoroughly. However, the authors note that this may not be the only, or even the main, reason for the genotype-habitat area effect, since *Pgi* is likely to influence many different aspects of life history.

The results of this study confirm that, under the right circumstances, intraspecific genetic variation can influence population growth. But they also make an important point about fitness. A major goal of evolutionary physiology is to understand the selective advantage of the traits found in a population, and it is often tempting in this pursuit to assume there is a single “best” genotype. This study provides a strong counterargument against such one-size-fits-all models of evolution, pointing out that in the ecological theater, which script plays best is a function of exactly which stage you are on.

Hanski I, Saccheri I (2006) Molecular-level variation affects population growth in a butterfly metapopulation. DOI: 10.1371/journal.pbio.0040129

Making the Connection: Polar Sites Are Key to Assembly in Many Immune Receptors

Mary Hoff | DOI: 10.1371/journal.pbio.0040168

Your ability to survive a paper cut or conquer a cold depends on immune system cells that circulate through your body, carrying out search-and-destroy missions against bacteria, viruses, and anything else that's not you. Such cells bear invader-snagging transmembrane proteins called receptors. When a receptor latches onto an invader (or a cell overtaken by invaders), it sends a signal through an associated signaling module. The complex then transmits a "got one!" message to the interior of the cell, where it elicits a full-scale defensive response.

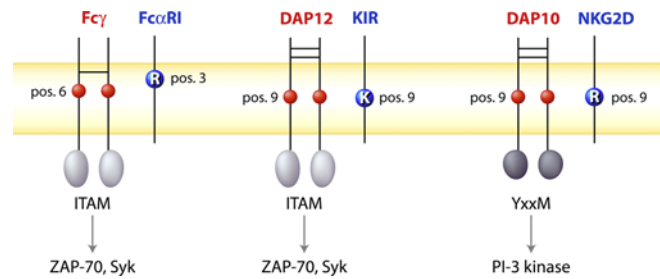
Proper assembly of receptors with the right signaling modules is key to an appropriate immune response, and incorrect assembly could well be a factor in immune system disorders such as chronic inflammation and autoimmunity. Jianwen Feng, Matthew E. Call, and Kai W. Wucherpfennig report fascinating findings about receptor assembly that shed light on how signaling modules can be versatile yet appropriately specific.

The researchers looked at the assembly of signaling modules with receptors from two key protein families, immunoglobulins and C-type lectins. The receptors they studied were KIR, NKG2D, NKG2C/CD94, and Fc α RI. The signaling modules under study were DAP10, which assembles with only NKG2D; DAP12, which assembles with KIR and many other receptors; and Fc γ , which assembles with Fc α RI and other receptors as well.

Previous research had shown that the assembly of receptors and signaling modules often involves attraction between one basic amino acid residue in the transmembrane part of the receptor and two acidic residues in the transmembrane part of the signaling module. The diversity of receptors that assemble with DAP12 and the wide variation among species in the amino acid makeup of DAP12 suggest that much of the rest of the transmembrane portion is less important for assembly. To test that, the researchers replaced all of the transmembrane residues in KIR (an immunoglobulin) with polyvaline or polyisoleucine except the one basic amino acid; they found it still assembled with DAP12 as long as the key acidic residues (both aspartic acid) of DAP12 had not been altered. If they replaced an aspartic acid, however, assembly was impaired. The authors also tested the altered KIR molecule in an actual cell and found that neither assembly of KIR nor its transport to the cell surface was prevented by the substitution.

Is the singular importance of the acid–base attraction for assembly true for other receptors as well? The researchers found that it is for the assembly of NKG2D, a C-type lectin, with DAP10. The same held true in the case of the assembly of the NKG2C portion of the NKG2C/CD94 receptor with DAP12 and for the assembly of Fc α RI with Fc γ (although in the latter case, assembly was reduced).

Why, with the ubiquity of this assembly mechanism, don't receptors and signaling modules end up making inappropriate matches? The base used to make the connection, the researchers found, is one key. KIR uses lysine, while Fc α RI and NKG2D use arginine. When the authors tried switching lysine for arginine or vice versa for KIR and Fc α RI—or tried to get the signaling modules to associate with each other's receptor—assembly failed.



Many activating receptors in the immune system assemble with their dimeric signaling modules in the membrane through an interaction between their basic transmembrane residue and a pair of acidic transmembrane residues of the signaling module.

(NKG2D assembly with DAP10 wasn't much affected, though, by switching out arginine and lysine.) Other tests showed that the position of the basic amino acid, the size and shape of the part of the receptor that sticks out of the cell membrane, and the different affinities of various signaling modules for various receptors also contribute to success in making the right receptor–signaling module match.

The bottom line? The mechanism by which immune system receptors and signaling modules assemble is similar enough among molecule types to allow some signaling modules to hook up with a wide range of receptors. At the same time, the specificity of the bases within the membrane, the shape of things above the membrane, and the differential ease of assembly of signaling modules provides needed specificity to prevent inappropriate assembly.

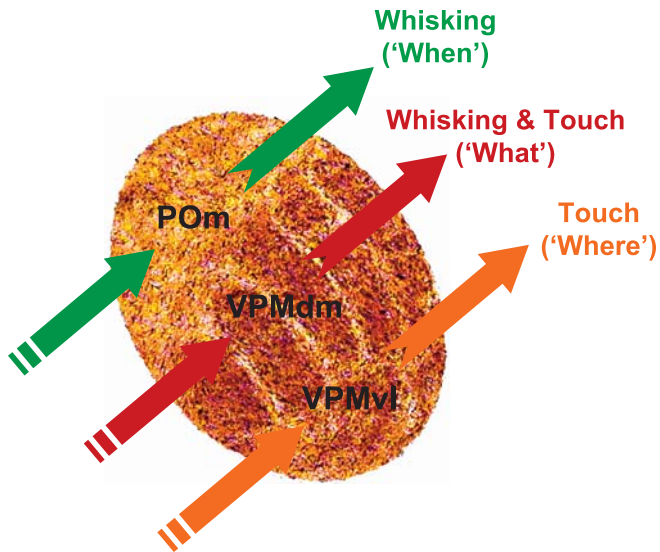
Feng J, Call ME, Wucherpfennig KW (2006) The assembly of diverse immune receptors is focused on a polar membrane-embedded interaction site. DOI: 10.1371/journal.pbio.0040142

Motion, Contact, or Both: Three Paths Convey Whisker Sensation in the Rat

Richard Robinson | DOI: 10.1371/journal.pbio.0040153

In the rat, whiskers are highly specialized sensory organs that serve much the same function as eyes and hands do for humans—they are a prime source of tactile and spatial information. The study of whisker sensory processing has proven fruitful for understanding how information is received, delivered, processed, and acted on in the brain. In this issue, Chunxiu Yu, Ehud Ahissar, and colleagues present novel insights into the connection between brain structure and sensory function by showing that whisker sensations that enter the thalamus, a central gateway of the brain, travel by three distinct pathways—two of which convey signals representing specific aspects of the sensory experience, while the third conveys a complex signal.

It has been shown previously that these three pathways, called the paralemniscal, extralemniscal, and lemniscal, carry



DOI: 10.1371/journal.pbio.0040153.g001

Three parallel trigeminal pathways through the thalamus convey whisking, contact, and whisking-touch signals, enabling parallel processing of sensor motion (“when”), object location (“where”), and object identity (“what”) in the whisking rat.

whisker sensations from sensory neurons via the thalamus and on to higher sensory-processing centers of the brain, but how these pathways handled the different types of information was unclear.

To examine this, the authors stimulated the facial nerve in anesthetized rats, causing the whisker to move as it does when the rats are actively moving their whiskers to explore the environment, a behavior known as “whisking.” Sometimes the whiskers contacted a rod placed in its path, while other times they contacted nothing. To see if the whiskers convey a different message depending on whether the rat is whisking versus when objects passively come into contact with the whisker, the authors also brought the rod in contact with stationary whiskers.

Using single-cell recording electrodes implanted in different sections of the thalamus, the authors could compare

the signals sent by the sensory neurons under these various conditions. They found that whisker movement induced activity in the paralemniscal pathway, whether or not the whisker touched the rod. Contact with the rod induced activity in the extralemniscal pathway, whether or not the whisker moved. And when the moving whisker contacted the rod, both pathways were active, along with the third pathway, the lemniscal.

The authors propose that the thalamic pathways function somewhat in parallel, each specialized for handling unique dimensions of movement and touch. In this arrangement, the paralemniscal handles temporal information related to motor control of whisking, the extralemniscal conveys object location, and the lemniscal pathway integrates a higher dimension of temporal and spatial information. The authors note that each of these pathways conveys information back to the motor nuclei by a different route, and thus is involved in a unique motor-sensory-motor loop. The authors caution, however, that these loops would not function in isolation, but, instead, can be considered parallel loops, with the higher processing loops building on the lower ones.

These results strengthen a model of the nervous system in which each sensory-motor pathway evolved in steps over time, with each new addition reaching to higher brain regions and subserving novel behaviors. In this scheme, evolution of movement sensation of the whiskers, conveyed by the paralemniscal pathway and processed in low brain regions, would have arisen first. This would be followed by evolution of contact detection, conveyed by the extralemniscal pathway and processed higher up in the brain to analyze object location. Finally, as analysis of object identity required greater detail, the lemniscal pathway would arise to convey the integrated information for higher brain analysis. Further testing of this model of nested motor-sensory-motor loops in this and other sensory systems may help determine the principles of active sensation.

Yu C, Derdikman D, Haidarliu S, Ahissar E (2006) Parallel thalamic pathways for whisking and touch signals in the rat. DOI: 10.1371/journal.pbio.0040124

Notch It Up: Nudging Stem Cells toward a Neural Fate

Liza Gross | DOI: 10.1371/journal.pbio.0040146

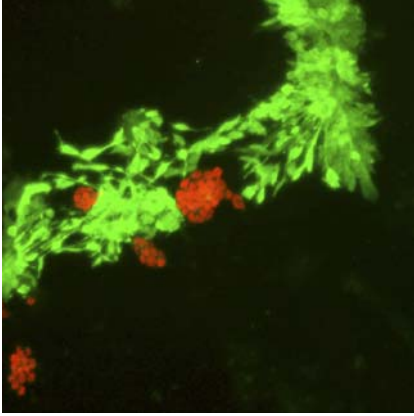
From the black widow spider to the six-toed sloth, every multicellular organism starts life as a single cell. This cell and the embryonic stem cells it spawns will live or die, grow, proliferate, migrate, and differentiate at the direction of a tightly controlled genetic program. Embryonic stem cells can self-renew to produce populations of identical cells that retain the ability to turn into any cell type of the body, a feature called pluripotency. Identifying the molecular signals that govern the maintenance and release of the pluripotent state would help scientists refine their ability to use embryonic stem cells

as models of disease, as test beds for drug screening, and as a source for cell-based therapies. Researchers are particularly interested in uncovering the early signals that commit a cell to a particular fate, such as a skin, gut, or nerve cell.

Cell fate is determined in a wide variety of vertebrates and invertebrates by proteins called Notch receptors, which straddle the membrane of cells and transmit signals through local cell interactions. Depending on the context, Notch signaling can inhibit the spread of differentiation among adjacent cells or prompt them to

adopt similar fates. In a new study, Sally Lowell, Austin Smith, and their colleagues discovered that Notch signaling also induces embryonic stem cells to make the initial commitment to a nervous system fate.

Working with undifferentiated mouse embryonic stem cells, Lowell et al. first confirmed that the cells express both the Notch receptor and its activators, or ligands. When the Notch pathway is activated, the receptor’s intracellular domain (NotchIC) detaches and enters the nucleus. Once inside the nucleus, NotchIC binds to and activates the RBPJ κ transcription factor, which in



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Neural progenitors (green) were efficiently generated from embryonic stem cells (red) through activation of the Notch pathway.

turn activates target genes. To boost Notch signaling without having to depend on complex ligands, the researchers engineered a transgene to allow ongoing, moderate expression of active NotchIC. Cells that showed this constitutive NotchIC expression, termed R26NotchIC cells, served as the experimental model. These cells come from an embryonic stem cell line (called 46C) that has green fluorescent protein linked to a neural specification marker gene (*Sox1*), making it easy to identify cells that choose a neural fate.

With a suitable experimental system in hand, Lowell et al. released R26NotchIC cells and a control cell line from the influence of self-renewal factors in order to allow differentiation. By the second day, they saw a roughly 3-fold increase in glowing *Sox1*-expressing cells compared with the control line. The researchers also observed reduced levels of a key marker of pluripotency (Oct4) and sharp increases of a protein associated with the initial stages of differentiation (FGF5). These findings, along with the observation that R26NotchIC cells give rise to a coherent mass of glowing *Sox1* cells, indicate that NotchIC accelerates the onset of neural differentiation. The researchers go on to show that Notch not only guides cells into a neural fate—amplifying and coordinating induction within a cell population—but also restricts them from choosing other fates.

To investigate whether NotchIC is necessary for neural induction, the researchers interfered with Notch activity. When 46C cells were treated with an agent that blocks Notch activation by preventing cleavage of its intracellular domain, the number of cells that activated the *Sox1* neural marker was reduced to only 10% of normal, and most retained expression

of the Oct4 pluripotency marker. Eventually, the cells differentiated, but not into neural cells. When this inhibitor was used to treat R26NotchIC cells, which have the already-cleaved receptor, *Sox1* expressed was unaffected; thus, the anti-neural effects come specifically from blocking Notch signaling. Using embryonic stem cell lines without functional *RBPJK* genes (needed to activate Notch's target genes) produced similar results: the cells yielded far fewer *Sox1* cells and either retained Oct4 or differentiated into a non-neural cell type.

Finally, Lowell et al. tested Notch's effects in human embryonic stem cells and show that it works much like it does in mouse stem cells, guiding them toward a neural fate. By revealing an unexpected role for Notch in directing early differentiation, the researchers have identified a key molecular determinant of stem cell regulation. As scientists identify more and more of these critical molecular cues, the closer they will come to harnessing the power and promise of these much-embattled, protean cells.

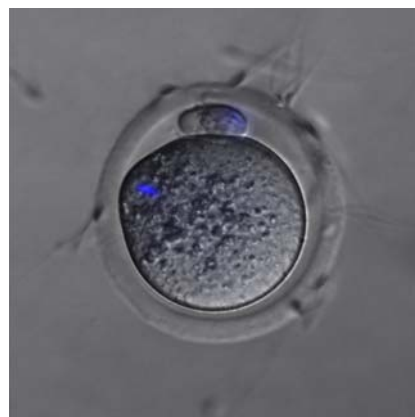
Lowell S, Benchoua A, Heavey B, Smith AG (2006) Notch promotes neural lineage entry by pluripotent embryonic stem cells. DOI: 10.1371/journal.pbio.0040121

The Ins and Outs of Sperm Entry

Françoise Chanut | DOI: 10.1371/journal.pbio.0040160

Egg cells have simple shapes, yet they have built-in asymmetries that can profoundly affect early steps in development. The football-shaped egg cell—or oocyte—of fruit flies contains a precisely laid mosaic of maternal molecules with the potential to induce different cell fates. As the fertilized oocyte divides, the resulting cells inherit distinct sets of maternal components, which endow them with different abilities to form head or tail, gut, muscles, or nerves. The spherical egg of frogs is also divided into areas that shift cells toward gut, muscle, or neural fate.

By contrast, the mammalian oocyte seems devoid of a clear blueprint for cell fates. All embryonic cells remain equally suited to give rise to all tissue types until implantation, when the embryo has divided several times. The general consensus is that in mammals, embryonic cells decide their fates by



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The first polar body (the smaller cell atop the oocyte) deforms the mammalian egg away from its encapsulating zona pellucida, creating a gap.

interacting with their neighbors, and not by a preset program handed down by the oocyte.

Still, a mammalian oocyte is not as simple as its roughly spherical shape suggests. For instance, its chromosomes hang close to its membrane, rather than at its center, and define a special area where some maternal molecules congregate. In addition, the oocyte remains in close contact with its sister cell from an earlier division, a far smaller cell called the first polar body. Both the chromosome area and the polar body are focal points that could, in theory, generate informative asymmetries. Such asymmetries may later influence which cells become the embryo versus the placental layers, or which cells initiate gastrulation movements. Watching the fertilization of mouse oocytes, Davor Solter, Takashi Hiiragi, and colleagues reported in a previous study that sperm enters the oocyte membrane preferentially in the hemisphere closest to the first polar

body. A simple explanation is that the oocyte membrane is asymmetric, the hemisphere near the polar body harboring more receptors for sperm than the other. But now, after further experiments, Nami Motosugi, Solter, Hiiragi, and colleagues have come to a different conclusion.

When they removed the mouse oocytes from their protective envelope, a soft shell called the zona pellucida (ZP), the researchers found that sperm cells entered with equal frequency through both halves of the oocyte. This dispelled the notion of an asymmetrically distributed sperm receptor. The only exception was a small area lying above the oocyte's chromosomes, a taut patch of membrane that appears refractory to sperm entry.

Inside an intact ZP, the polar body presses against the oocyte, which locally increases the space between oocyte and ZP—the so-called perivitelline space (PVS). The authors

reasoned that this local increase of PVS volume might be responsible for the sperm entry bias. Indeed, by simply enlarging the PVS all around the oocyte, either by removing half of the cell's content or by inserting a second polar body opposite the original one, they were able to significantly increase the frequency of sperm entry events far from the first polar body.

Using time-lapse videos and careful marking strategies, the researchers showed that sperm broke through the ZP at random locations, and could swim frantically for a few minutes before entering the oocyte. Once random motion brings a sperm cell near the polar body, the likelihood is high that it will remain there and pierce the oocyte membrane nearby, simply because of the additional swimming space afforded by the expanded PVS. This simple spatial asymmetry is sufficient, the authors argue, to explain the bias they observed in the choice of sperm entry

site, without invoking any asymmetry of the oocyte membrane.

Aside from providing further evidence that the mammalian egg cell is unpatterned, these observations also support the validity of in vitro fertilization practices that introduce sperm more or less randomly directly inside the oocyte.

As to why sperm should normally spend several minutes pacing the PVS before fertilization, the authors suggest that this gives it time to complete the maturation that allows its fusion with the oocyte. That sperm should waver in the egg's antichamber before tying the knot will come as no surprise to anybody who has ever faced such a momentous decision.

Motosugi N, Dietrich JE, Polanski Z, Solter D, Hiiragi T (2006) Space asymmetry directs preferential sperm entry in the absence of polarity in the mouse oocyte. DOI: 10.1371/journal.pbio.0040135

Conformational Variations of a Key Enzyme Offer Clues to Cancer-Drug Resistance

Mary Hoff | DOI: 10.1371/journal.pbio.0040166

The smooth operation of a cell requires that critical changes in molecular activity take place when and where they're supposed to. One family of enzymes, called tyrosine kinases, plays an important role in effecting these changes by attaching phosphate groups to other proteins in a process called phosphorylation. This chemical reaction initiates a tag team of signals that lead from a stimulus, through a series of intervening molecules, to a protein controlling a specific cellular process. To phosphorylate the recipient protein, a tyrosine kinase must first grab onto it with a highly specific lock-and-key connection—each kinase tailored to its unique target. This happens only when the kinase is in an active conformation—when the key has been twisted or bent by a signal from the previous molecule in the tag team into a conformation that will fit the shape of its target protein.

C-Abl is a tyrosine kinase that helps convey messages about cell growth and movement. Normally it does so only when it is activated by the preceding protein in the signaling pathway. But when cells possess a mutated form of c-Abl called BCR-Abl, the tyrosine kinase gets stuck in its active form, and sends the cell constant encouragement to grow. The miscommunication manifests itself as the blood cancer chronic myeloid leukemia, or CML.

A common treatment for CML is a drug called imatinib (known commercially as Gleevec or Glivec). Imatinib binds to a conformation of Abl (either c- or BCR-) in which a section called DFG has been rotated about 180 degrees from the active form, "DFG-Asp In," to the inactive "DFG-Asp Out" conformation, preventing the tyrosine kinase from doing its phosphate-transfer job. But in some CML cells, imatinib doesn't work. A better understanding of the various



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Simulations of the Src-like inactive conformation of Abl.

conformations of the kinase domain of Abl will shed light on not only the molecule's function, but also how imatinib does—and sometimes doesn't—inhibit it.

Nicholas M. Levinson, Olga Kuchment, John Kuriyan, and colleagues explored the ins and outs of the kinase

portion of normal and imatinib-resistant (mutant) Abl using crystallography, a process that makes it possible to take “snapshots” of proteins in various conformations. With the use of a novel synthetic bisubstrate analog inhibitor, the researchers found four previously undescribed forms of the kinase domain of Abl. They analyzed the position of various key amino acids and groups of amino acids within these conformations, as well as within a fifth, already-known conformation: that of Abl with imatinib attached.

In doing so, the researchers uncovered a big surprise: an inactive Abl conformation that differs dramatically from the rest of the conformations studied—and from all previously known conformations of Abl. This unprecedented inactive conformation was very similar to the inactive form of another kinase called Src, in which DFG is not flipped but another part of the kinase, an α helix, is swung out from the active conformation, known as α C-Glu In, into the inactive form, α C-Glu Out.

The researchers explored the functional significance of the odd inactive conformation using clues garnered

from the other conformations they observed and from sophisticated computer simulations that allowed them to model changes from one configuration to another. The results of the simulations supported their speculation that the Src-like form might be an intermediate that facilitates the DFG flip.

Could this be a clue to imatinib resistance? Some forms of imatinib resistance are known to result from mutations that block the binding of imatinib to Abl. More commonly, however, mutations prevent Abl from adopting the conformation that imatinib binds to. The researchers noted that such imatinib-resistant mutations tend to destabilize the Src-like conformation of Abl more than the active or imatinib-bound conformations of Abl, suggesting that this conformation does indeed play a role in whether imatinib is effective in blocking the activity of Abl in CML cells.

Levinson NM, Kuchment O, Shen K, Young MA, Koldobskiy M, et al. (2006) A Src-like inactive conformation in the Abl tyrosine kinase domain. DOI: [10.1371/journal.pbio.0040144](https://doi.org/10.1371/journal.pbio.0040144)