

Synopses of Research Articles

Neandertals Likely Kept Their Genes to Themselves

Scientists searching for clues to our origins have long relied on studying fossils to piece together our evolutionary history. Now, with the tools of molecular genetics, they can reach beyond morphological evidence to retrieve traces of DNA preserved in the remnants of bone. And in these ancient DNA sequences, they're finding bits and pieces of the evolutionary record. Over the course of evolution, changes in DNA sequences accumulate at a predictable rate. These mutations can reveal not only how closely related we are but also when evolutionary lineages diverged. Identifying both a typical range of genetic variation and rate of mutation for a given species or population, for example, can serve as a frame of reference for analyzing DNA sequences from other species or populations. Most molecular anthropologists use DNA found in mitochondria—intracellular structures that convert food into energy—to reconstruct human evolution. Distinct from nuclear DNA, mitochondrial DNA (mtDNA) exists in the cytoplasm of a fertilized egg and is passed on only through the maternal lineage.

An ongoing debate about human origins has revolved around the theory that *Homo sapiens* and *Homo Neanderthalensis* interbred, since the two species coexisted. Neandertals lived roughly 150,000 to 30,000 years ago, toward the end of the Pleistocene era, and inhabited Europe, parts of Asia, and the Middle East. Modern-day humans arose between 100,000 and 200,000 years ago. Recently, an international multidisciplinary team of scientists led by Svante Pbo of the Max Planck Institute for Evolutionary Anthropology have analyzed the largest sample of Neandertal and early human remains to date and conclude that Neandertals

could not have made a significant genetic contribution to early modern humans.

Part of the challenge of resolving the human–Neandertal interbreeding issue stems from the fact that so many fossil samples—of both early humans and more archaic humans—are contaminated with the DNA of the contemporary humans who have handled them. So even if a Neandertal sample contained a “real” (or endogenous) DNA sequence resembling early humans—which



Neandertal skull from La Chapelle aux Saints

would indicate intimacy between the two groups—it might be considered contaminated. When Pääbo and colleagues looked for modern DNA, they found it in every sample they examined: in the Neandertal and early human fossils—and even in cave bear teeth. To circumvent this problem, they looked only for Neandertal mtDNA as evidence of interbreeding. Since it is easy to distinguish modern human mtDNA sequences from the four Neandertal mtDNA samples that have been sequenced so far, the researchers decided to determine whether Neandertal-like mtDNA could be found in other Neandertal fossils as well as in early human remains.

As these fossils are precious commodities, Pbo's group applied a technique developed in their lab that uses amino acid content as a measure of extractable endogenous DNA and requires removing just 10 mg of bone from a specimen rather than much larger pieces of bone. Of 24 Neandertal and 40 early modern human fossils analyzed, they found four Neandertal and five early human specimens that passed the amino acid test. These fossils

included samples classified as “transitional” between the two groups and represented a wide distribution across Europe, where the two groups would likely have encountered one another. When they analyzed these samples for Neandertal mtDNA, they found mtDNA sequences that are absent in contemporary human mtDNA genes but quite similar to those found in the four previously sequenced Neandertals. They found no Neandertal-like mtDNA in the early human samples.

While the authors explain that it's impossible to definitively conclude that no genetic flow occurred between

early humans and Neandertals given the limited number of early human fossils available, they point out that even fossil samples considered as anatomically transitional between modern humans and Neandertals failed to show evidence of mtDNA exchange. Thus, Pääbo and colleagues conclude, while it's possible that Neandertals made a small contribution to the genetic makeup of contemporary humans, the evidence cannot support the possibility of a large contribution.

Serre D, Langaney A, Chech M, Teschler-Nicola M, Paunovic M, et al. (2004) No evidence of Neandertal mtDNA contribution to early modern humans. DOI: 10.1371/journal.pbio.0020057

Conserved Genes Preferentially Duplicated in Evolution

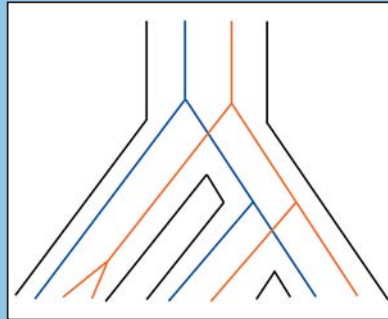
Over the course of evolution, some organisms have gained many genes and become increasingly complex whereas other, simpler, organisms have survived with comparatively fewer genes. (Compare, for example, the 30,000 genes of humans to the 5,500 of brewer's yeast.) But where do these "new" genes come from? Evolutionary biologists have long known that duplication of existing genes is an important source of genetic novelty—it is easier to copy and modify an existing gene than to create a completely new one from scratch.

Because gene duplication makes such a major contribution to evolution, researchers have attempted to understand the mechanisms of gene duplication, how genes evolve once they become duplicated, and what functional effect gene duplications have for the organism. Recent genomic studies, for example, appear to show that most duplicated genes go through a period of accelerated evolution and also that the presence of duplicated genes adds robustness to the functioning of genomes. In research published in this issue, however, Jerel Davis and Dmitri Petrov look at gene duplication from a different perspective. Rather than asking how genes are duplicated, they asked which genes tend to be "good" at duplicating over the course of evolution. The answer is important for our understanding of the forces underlying gene duplication and will also help us understand why genomes contain duplicates of some genes and not others.

The authors began by identifying duplicated and nonduplicated gene pairs in the yeast *Saccharomyces cerevisiae* and the worm *Caenorhabditis elegans*, two model organisms whose genomes have been sequenced. They then looked for the corresponding genes in two distantly related species, the fruitfly and the mosquito, in order to obtain an independent measure of evolutionary rate. This independent measure is vital

because of the likelihood that gene duplication itself influences the rate of evolution. After obtaining these rates, the researchers compared the evolutionary rates of duplicated and nonduplicated genes.

Stated simply, the authors found that slowly evolving (that is, more conserved) genes are more successful at generating duplicates than faster evolving genes. This is no recent trend—more conserved



Phylogenetic studies show that slowly evolving genes are more likely to be duplicated than faster evolving genes

genes have been better at generating duplicates of themselves consistently over hundreds of millions of years.

This research challenges the assumption that genes are duplicated in an unbiased manner. In addition, it provides the essential background for other genomic

studies of gene duplication. For example, the acceleration of protein evolution upon duplication is likely to be even more dramatic considering that it is the slowly evolving genes that duplicate preferentially.

These findings also open up new questions in the study of gene duplication. The authors convincingly demonstrate the bias toward conserved genes in the process of duplication, but how and why does this happen? For a duplicated gene to be retained in a species, the duplicate must be fixed in the population and then must be preserved by natural selection. The preferential duplication of slowly evolving genes might come from a bias in either of these steps, and the authors outline several models for why this might be the case. Further analysis may enable researchers to test these and other models for gene duplication—especially as more sequence data become available—and learn more about this potent phenomenon in genome evolution.

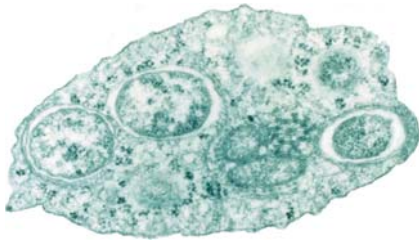
Davis JC, Petrov DA (2004) Preferential duplication of conserved proteins in eukaryotic genomes. DOI: 10.1371/journal.pbio.0020055

Genome Sequence of the Intracellular Bacterium *Wolbachia*

Wolbachia have a thing against males. A member of one of the most diverse groups of bacteria, called Proteobacteria, this parasitic "endosymbiont" lives inside the reproductive cells of a wide variety of the nearly 1 million species of arthropods, including insects, spiders, and crustaceans. It has also been found in worms. *Wolbachia*'s preferred habitat is the cytoplasm of its host's gametes. Since sperm have very little cytoplasm, *Wolbachia* seek out the company of females, securing its survival by hitching a ride to the next generation in the cytoplasm of the mother's eggs. *Wolbachia*'s effects range from beneficial to pathological, depending on which species infects which invertebrate host, but since most species are not beneficial, *Wolbachia* infections often turn out badly if the host is male. On the other hand, if female, the host could very well live longer, produce more eggs, and have higher hatching rates than its noninfected cousins—thereby facilitating *Wolbachia*'s transmission from mother to offspring.

Wolbachia have evolved an impressive repertoire of "reproductive parasitic" strategies to adapt its host's physiology to its own advantage. One strategy involves inducing "cytoplasmic incompatibility" between sperm and egg, which in effect uses infected males to keep uninfected females from producing viable offspring. Another causes infected females to reproduce asexually, creating a new generation of infected clones. Another turns developing male embryos into females. And, in a pinch, some *Wolbachia* simply kill developing males. The biochemical mechanisms that trigger different strategies in different hosts are unclear, however, in part because it's so far been impossible to cultivate sufficient quantities of these obligate endosymbionts (that is, intracellular species that cannot survive outside their host). But now that Scott O'Neill, Jonathan Eisen, and colleagues have sequenced the complete genome of one strain of *Wolbachia pipientis*, scientists investigating the biology and evolution of *Wolbachia*–host interactions have a valuable new research tool. The strain they sequenced, *W. pipientis* wMel,





Transmission electron micrograph of *Wolbachia* within an insect cell
(Image courtesy of Scott O'Neill.)

lives inside the fruitfly *Drosophila melanogaster*, the favorite model organism of geneticists for nearly 100 years. This strain causes cytoplasmic incompatibility in its host.

The structure of the *wMel* genome, the O'Neill and Eisen groups note, is strikingly different from any other obligate intracellular species. While its genome is compact, it nonetheless contains large amounts of repetitive DNA and "mobile" DNA elements. Mobile genetic elements, as the name implies, are DNA sequences that move around the genome and are often acquired from other species. Most of the repetitive and mobile elements in *Wolbachia* do not appear in other α -Proteobacteria species and were probably introduced some time after *Wolbachia* split off from its evolutionary ancestors. *Wolbachia*, unlike

other obligate intracellular bacteria, seem quite amenable to incorporating foreign DNA, which the authors speculate was introduced by the bacteria-infecting virus called phage.

Analysis of the *Wolbachia* genome sheds light on the mechanisms that might help the parasite manipulate the host cell's physiology to its own advantage. One likely bacterial weapon for host exploitation is the abundance of predicted genes encoding ankyrin repeat domains, amino acid sequences characteristic of proteins important for protein-protein interactions in eukaryotes (organisms with nuclei, which bacteria lack). In bacteria, ankyrin repeats might regulate host cell-cycle pathways, which one wasp-infecting *Wolbachia* strain modifies to induce cytoplasmic incompatibility. Other molecular interactions between *wMel* and its host, the researchers propose, might also rely on proteins with these ankyrin repeats.

The *Wolbachia* genome also provides insight into mitochondrial evolution. It is widely believed that these intracellular energy-metabolizing centers were once free-living bacteria belonging to the α -Proteobacteria group, though it's not clear which branch of the α -Proteobacteria tree they inhabit. Complete genome analysis of various α -Proteobacteria—including *wMel*, the

first non-*Rickettsia* species sequenced in the Rickettsiales group—provides no evidence that mitochondria are more related to *Rickettsia* species than to *Wolbachia*, as was previously thought. In fact, further analysis failed to consistently connect mitochondria to any particular species or group within the α -Proteobacteria.

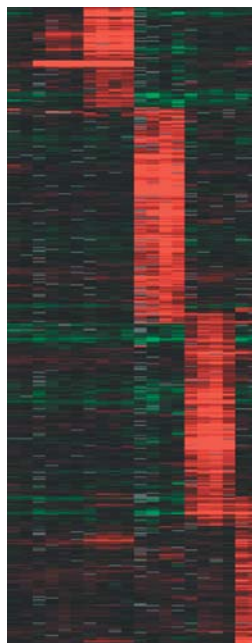
While the information hidden in the *Wolbachia* genome seems to raise as many issues as it settles, biologists studying a wide range of problems—from the evolution and biology of *Wolbachia* and endosymbiont-host interactions to the origin of mitochondria—have a valuable new tool to explore their questions. The *Wolbachia* genome will also provide important molecular guidance for efforts to suppress insect pests and control filariasis, a human disease caused by worms. Since beneficial *Wolbachia* live in both insect and worm, applying antibiotics to target the *Wolbachia* will ultimately kill the insect pest and infecting worm, which both depend on the bacteria to survive.

Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, et al. (2004) Phylogenomics of the reproductive parasite *Wolbachia pipientis* *wMel*: A streamlined genome overrun by mobile genetic elements. DOI: 10.1371/journal.pbio.0020069

mRNA Targets of RNA-Binding Proteins Suggest an Extensive System for Post-Transcriptional Regulation

The single-celled *Saccharomyces cerevisiae*, commonly known as baker's yeast, measures just 2 microns—it takes about 4 billion to fill a teaspoon. But as a eukaryote (its cells have nuclei), its genes function in much the same way a human's do. For a gene to function, its DNA sequence must first be transcribed into RNA (called messenger RNA, or mRNA), whose sequence can then be translated into a specific string of amino acids to form the unique protein that the gene encodes.

The population of mRNA transcripts in each cell (its "transcriptome") is dynamic—the genome uses its vocabulary of genes to write an ever-evolving script for the cell as its life unfolds and its environment changes. By binding to specific sequences of DNA, proteins called transcription factors process signals from the cell's sensory and information-processing systems



Cluster of RNA targets for Puf proteins

to control which genes are transcribed in each cell, under what conditions, and at what rate. While the steps and regulatory programs that govern gene expression at this level are reasonably well known, much less is known about the orchestration of the later steps in the gene expression program—where in the cell each mRNA molecule goes when it leaves the nucleus, at what rate and under what conditions it is translated into protein, and how long it survives.

RNA-binding proteins (RBPs) have been implicated in diverse aspects of post-transcriptional gene regulation. Hundreds of RBPs are encoded in the eukaryotic genome, but because few have been studied in detail and few of their mRNA targets are known, the nature and extent of an RBP-mediated post-transcriptional program has been obscure. Now a systemic analysis of a specific family of RBPs and their mRNA

targets in yeast by André Gerber, Daniel Herschlag, and Patrick Brown, of Stanford University, suggests that such a program may exert detailed control over the life history of every mRNA. By selectively binding and regulating specific classes of mRNAs, RBPs may provide a mechanism to coordinate the collective fate of these transcripts and serve as an integral part of the global transcriptome.

Gerber, Herschlag, and Brown focused on the binding targets of a family of RBPs called Pumilio-Fbf (Puf) proteins, which are defined by the presence and configuration of an amino acid domain that mediates RNA-binding. Little is known about the physiological function of the five yeast Puf proteins the researchers studied here (called Puf1p–Puf5p). After using “affinity tags” to snag each of the five Puf proteins from yeast cells, together with their bound mRNA targets, the researchers identified the associated mRNAs with microarray analysis. They found more than 700 mRNAs bound by at least one Puf protein,

with each Puf RBP targeting a distinct group of mRNAs. The group of mRNAs associated with each Puf protein turned out to encode proteins with strikingly similar functions and locations in the cell. Many of the mRNA sets encode proteins that reside in the same cellular location, are part of the same protein complexes, or act in the same signaling pathway. Some Puf proteins target mRNAs that encode membrane proteins while others preferentially bind to mRNAs that encode proteins involved in cell division. The most pronounced bias occurs with Puf3p, which overwhelmingly binds mRNAs that encode proteins destined for the mitochondria, the cell’s power generators.

This selective tagging of functionally related mRNAs by specific RBPs suggests a mechanism for coordinated global control of gene expression at the post-transcriptional level. Just as transcription factors regulate transcription by binding to specific DNA sequences, RBPs may mediate regulation of the subcellular localization, translation, and degradation

of the set of specific mRNAs they target. Noting the striking themes in the subcellular localization of the proteins encoded by the mRNAs bound by each Puf protein, Gerber, Herschlag, and Brown propose that RBPs may play important roles in the subcellular localization and efficient assembly of protein complexes and functional systems by ensuring that the location in the cell at which mRNAs are translated “is not left to chance.” Since the number of RBPs encoded in eukaryotic genomes approaches that of transcription factors, the regulatory program that controls the post-transcriptional fate of mRNAs—their localization, translation, and survival—may prove to be nearly as diverse and complex as the regulation of transcription itself.

Gerber AP, Herschlag D, Brown PO (2004) Extensive association of functionally and cytologically related mRNAs with Puf family RNA-binding proteins in yeast. DOI: 10.1371/journal.pbio.0020079

A Holistic Approach to Evaluating Cellular Communication Pathways

To function properly, cells must keep constant tabs on the environmental conditions around them, such as the presence of growth hormones in the blood or the proximity of neighboring cells. These external cues are relayed into the cell through a cascade of chemical and physical reactions referred to as signal transduction. Signal transduction pathways inform and regulate almost all activity within the cell, from protein production to cell division. Understanding these processes is fundamental to biology, but the sheer number of molecules and interactions in some pathways makes thorough documentation difficult.

Taking a holistic approach that combines both computational models and experimental manipulations, scientists have described the web of interactions involved in the aryl hydrocarbon receptor (AHR) signal transduction pathway. AHR belongs to the Per–Arnt–Sim (PAS) superfamily of sensor molecules that regulate functions like development, the sleep–wake cycle, and cellular reaction to oxygen deprivation. Unlike many receptors that are embedded in the cell membrane, AHR floats freely in the main body of the cell, called the cytosol. There it waits for a stimulus or

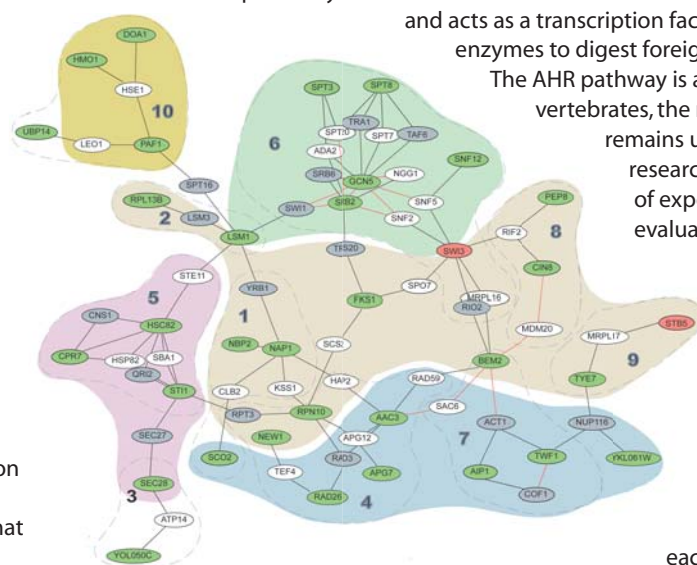
ligand, such as a dioxin molecule, to enter the cell and bind to it. Once bound, AHR undergoes a host of changes, glomming on to additional molecules before it enters the cell nucleus and acts as a transcription factor, initiating the production of enzymes to digest foreign, or xenobiotic, compounds.

The AHR pathway is a curiosity; though found in all vertebrates, the natural, or endogenous, ligand remains unknown. Without this knowledge, researchers are limited in the kind of experiments they can perform to evaluate the pathway.

Christopher Bradfield and colleagues used yeast as a model system to elucidate the steps involved in this pathway, which regulates vertebrate cell response to pollutants like dioxins. To first assess the molecules involved in the AHR pathway, the team used 4,507 yeast “deletion” strains, each strain missing one gene from its genome. They then inserted the AHR gene into the strains using small rings of movable DNA called plasmids. Though

yeast does not naturally possess AHR, it is an ideal genetic model for studying signaling pathways due to its quick generation time, small, well-characterized genome, and similarity to vertebrate systems.

Bradfield’s team exposed each strain to a receptor stimulus or agonist and screened them for AHR response. If a deletion strain showed significantly reduced activity, they concluded that



Protein-interactive-network for AHR signaling

the missing gene was a key component to the signal pathway. The researchers identified 54 genes that had a significant influence on AHR response. Only two of these genes, termed modifiers, had been previously identified.

Signaling pathways usually boil down to a series of discrete steps. To identify steps of the AHR pathway, the researchers constructed a spider web-like map called a "protein interaction network," or PIN, based on previously known interactions between the proteins encoded by the 54 modifier genes. The resulting map revealed groups of highly connected, related modifiers, which the authors proposed to be steps in the pathway. Though other studies have used the newly developed PIN strategy to investigate cellular processes, Bradfield's team also annotated their PIN through a series of experiments both to support the identity of and to better understand the protein groups, referred to as functional modules.

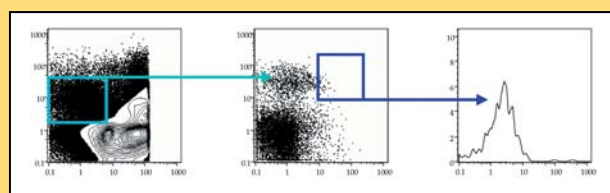
With tests based on discrete receptor signaling events, known active structural regions, reaction to different types and concentrations of agonists, and functional location within the cell, Bradfield's team organized the functional modules into five steps. One group of modifiers is involved in AHR folding, the conformational change that occurs when the receptor binds to a toxin. With the help of other modifiers, the new AHR complex is then translocated into the cell nucleus. Once in the nucleus, a series of modifiers assist the AHR in its role as a transcription factor. The researchers also identified a step in the pathway that controls production of AHR itself and another unknown "step" that takes place inside the nucleus.

As AHR is thought to be a prototype PAS receptor, understanding the steps in this pathway will likely guide future research on the entire family, allowing scientists to study in detail individual steps in these complex pathways. The highly integrated method reported here could also be used to study most other mammalian signaling pathways, giving scientists a new tool as they attempt to understand how cells respond to their changing environment.

Yao G, Craven M, Drinkwater N, Bradfield CA (2004) Interaction networks in yeast define and enumerate the signaling steps of the vertebrate aryl hydrocarbon receptor. DOI: 10.1371/journal.pbio.0020065

Tracking Blood-Forming Stem Cells through Development

Of the 200-plus different types of cells that form the mammalian body, most have a finite life span. Like nearly everything in biology, there are exceptions—neurons and muscle cells, for example, can last a lifetime—but the vast majority of cells eventually wear out and must be replaced. Among the most short-lived cells, blood cells are generated continuously, mainly in the bone marrow of an adult, recharging the bloodstream as their depleted predecessors are efficiently dispatched and removed from circulation every 120 days. Some 2.5 million new red blood cells are generated every second from a small pool of stem cells.



Fluorescence activated cell sorting is used to study the early development of hematopoietic stem cells

Blood cell development, called hematopoiesis, passes through discrete stages in specific tissues in the developing embryo before converging in the bone marrow, where it continues throughout adulthood. Some researchers have proposed that hematopoietic stem cells (HSC) flood the bloodstream during short, precise intervals to build the developing hematopoietic system (which includes the liver, bone marrow, spleen, tonsils, and lymph nodes). Presenting an alternative model for HSC migration, Julie Christensen and her colleagues in Irving Weissman's lab at Stanford University report that HSC in mice gradually leave the fetal liver to colonize the developing spleen and bone marrow as the organs acquire the means to support them.

In mouse embryos, HSC precursors develop first in the yolk sac and a region called the aorta-gonad-mesonephros (AGM), then they migrate to the liver, and later to the spleen, before finally settling into the bone marrow just before birth. It was thought that this migration occurs in distinct waves of HSC production because HSC numbers decrease in one region just before increasing in newly forming hematopoietic sites. Analyzing the concentration and activity of HSC, Christensen et al. found the cells in the blood at low but fairly constant levels

during much of late fetal development, when they migrate from the liver to the spleen and bone marrow. Although the HSC population decreases in the liver at 15.5 days after conception, the authors propose that this drop occurs primarily because the HSC have differentiated into mature blood cells, not because they've exited the liver en masse to help build the spleen and bone marrow. On the other hand, the slight decrease in circulating HSC, which also occurs around this time, may be attributed to their recruitment from the bloodstream to these developing tissues.

Christensen et al. also examined the impact of intercellular signaling proteins called chemokines, which help regulate fundamental developmental processes, on HSC migration. To effectively "seed" developing tissues, HSC must first be

recruited from the blood, guided to the appropriate nascent tissue, then corralled and sustained. The chemokine SDF-1 attracts and retains HSC in the bone marrow but was thought to have a lesser effect on fetal liver HSC. Christensen et al. demonstrate not only that liver HSC migrate in response to this chemokine, but that their migratory response increases dramatically when both SDF-1 and a signaling protein called steel factor (SLF) are present. While adult marrow HSC respond to SDF-1, they do not respond to SLF alone and do not show improved migration in the presence of both SLF and SDF-1.

Bone marrow transplants have become increasingly common for a number of hematological disorders, including leukemia and aplastic anemia. Since hematopoiesis occurs primarily in the bone marrow in both mice and humans after birth, these findings offer valuable insights into the migratory behavior of these stem cells and suggest how HSC migration might be applied to bone marrow transplants and other clinical therapies.

Christensen JL, Wright DE, Wagers AJ, Weissman IL (2004) Circulation and chemotaxis of fetal hematopoietic stem cells. DOI: 10.1371/journal.pbio.0020075

A Single Mutation Transforms an Iron Transporter into an Ion Channel

Trace heavy metals are essential for a number of metabolic reactions in living systems, but cells walk a fine line between feast or famine. While iron, zinc, cobalt, and manganese, for example, contribute to the catabolic activity of enzymes involved in essential pathways from gene regulation to cell signaling, even a mild surplus of these metals can kill cells and cause a variety of diseases. Maintaining the proper concentration, or homeostasis, of cellular metals requires strict policing of what passes through cell membranes and organelles.

One way cells regulate entry is through the hydrophobic lipid (fatty) layer that makes up the cell membrane. While the lipid membrane allows most small fat-soluble or uncharged molecules to simply diffuse through it, nearly all water-soluble molecules, including metal compounds—which typically break down into ions (molecules with positive or negative charge) in solution—rely on either transport or channel proteins to get through.

Two types of proteins manage the transport and uptake of iron ions in mammalian cells: the transferrin receptor helps to concentrate iron in discrete intracellular compartments called endosomes, while a protein called divalent metal transporter-1 (DMT1) releases iron into the cytoplasm, where it supports essential metabolic processes. DMT1 also serves to bring dietary iron directly into the intestinal cells involved in iron absorption. DMT1 preferentially carries iron, zinc, copper, and manganese, but not calcium. This selectivity helps strike the right balance of the concentration of these metals in the cell. Recent structural analyses of transporters, however, have raised the possibility that this selectivity may not be as fixed as once thought. Lending support to the notion that the distinctions between transporters and ion channels are blurring, David Clapham, Nancy Andrews,

and colleagues report that a mutation causing a single amino acid substitution in the DMT1 metal ion transporter opens a passageway that converts the transporter into a calcium channel.

DMT1 is essential for maintaining iron homeostasis and the only molecule known to facilitate transmembrane iron

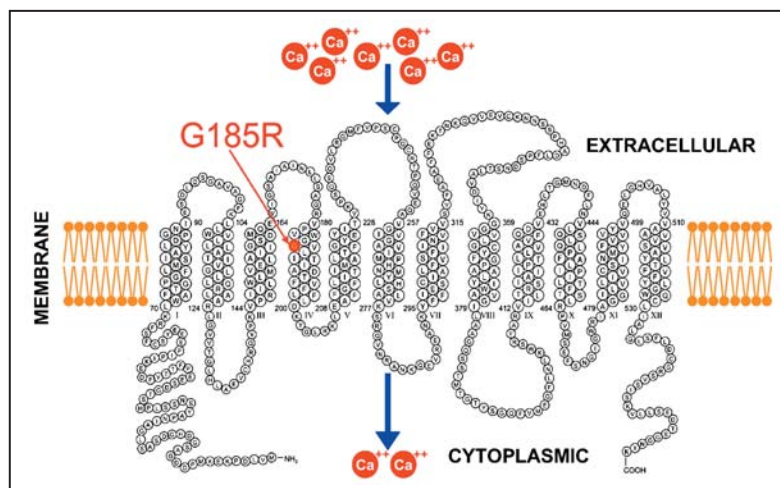
physiological effect on the mice with this mutation, the researchers compared the properties of intestinal epithelial cells taken from the mutant and nonmutant animals. The intestinal cells in the mutant mice showed high levels of the G185R protein and a large current of charged molecules—much as would occur in an ion channel. This current was observed in both the cell lines expressing G185R and the cells extracted from the G185R mutant mice.

The G185R mutation, the researchers conclude, appears to either expose or enhance a calcium “permeation pathway” that exhibits the properties of a calcium channel. This transformation appears to offer a selective advantage, since mice engineered without the DMT1 protein die within a week

of birth while mice born with the G185R DMT1 mutation can live for over a year. Though the G185R mice exhibit severe iron deficiency, the modest function retained by G185R in combination with the increased influx of calcium may be enough to extend their lifespan. The increased levels of calcium, the researchers propose, may support iron uptake through some other pathway, an advantage that might explain why such a mutation would persist.

Whatever mechanism accounts for this advantage, the G185R mutation transforms DMT1 transporter into an “unambiguous” calcium ion channel. Investigating the structural and biochemical properties of this molecular changeling will provide valuable insights into the emerging model of a transporter–channel continuum—which suggests a remarkable adaptability to shifting environmental conditions.

Xu H, Jin J, DeFelice LJ, Andrews NC, Clapham DE (2004) A spontaneous, recurrent mutation in divalent metal transporter-1 exposes a calcium entry pathway. DOI: 10.1371/journal.pbio.0020050



A single mutation of the amino acid glycine (G) to arginine (R) turns a membrane transporter into a calcium channel

uptake in higher eukaryotes, including humans. It is expressed mainly in epithelial cells of the small intestine, where iron metabolism is monitored, and in endosomes, which release transferrin-imported iron. The Clapham and Andrews groups focused on a mutation in the DMT1 transporter called G185R—which substitutes the arginine (R) amino acid for glycine (G) at a particular location in the protein’s amino acid chain, position 185—because the identical mutation has occurred spontaneously in three separate laboratory strains of rodents (two mouse and one rat strain). That a single substitution has arisen independently and persisted in multiple rodent generations suggests it may confer some type of selective advantage.

To investigate this idea, the researchers compared the properties of “wild-type” (nonmutant) DMT1 and mutant G185R in laboratory cell lines. They found that cells expressing G185R mutant proteins had much lower levels of iron uptake than cells expressing the nonmutant proteins, but that they also permitted the influx of calcium ions. To see whether the G185R-mediated calcium permeability had a

A Mechanism for Amphetamine-Induced Dopamine Overload

The notion of a neurally encoded “reward system” that reinforces pleasure-seeking behaviors first emerged fifty years ago. Psychologists James Olds and Peter Milner discovered this phenomenon when their “lack of aim” landed an electrode outside their target while studying the behavioral responses of rats given electrical stimulation to a particular brain region. It was known that stimulation of certain brain regions would induce an animal to avoid the behavior that produced the stimulus. But in the rat with the “misplaced” electrode, stimulation of this new region caused the rat to repeat the behavior that caused the stimulus. Stimulation of certain brain regions provides a very strong incentive to restimulate, creating a feedback loop that reinforces both the behavior and the neural response to it.

When gentle shocks were delivered to the rat hypothalamus, for example, the animals would “self-stimulate” 2,000 times per hour by pushing a lever. The neurotransmitter dopamine, it was later discovered, plays an important role in the brain’s reward system—and in laying the biochemical foundation of drug addiction.

Essential for normal central nervous system function, dopamine signaling mediates physiological functions as diverse as movement and lactation. The dopamine transporter (DAT) is involved in terminating dopamine signaling by removing the dopamine chemical messenger molecules from nerve synapses and returning them into the releasing neurons (a process called reuptake). DAT can also bind amphetamine, cocaine, and other psychostimulants, which inhibit dopamine reuptake, and, in the case of amphetamine, also stimulate the release of dopamine through DAT. It’s thought that abnormal concentrations of dopamine in synapses initiate a series of events that cause the behavioral effects of these drugs. The biochemical steps underlying amphetamine-induced dopamine release, however, are not well characterized. Now, a team led by Jonathan Javitch and Aurelio Galli has identified a chemical modification of DAT that is essential for DAT-mediated dopamine release in the presence of amphetamine. Since this modification does not inhibit the ability of DAT to accumulate dopamine, it may suggest a molecular target for treating drug addiction.

Embedded in the membrane of nerve cells, the dopamine transporter has a “tail,” called the N-terminal domain, that protrudes into the cell interior and consists of a stretch of about

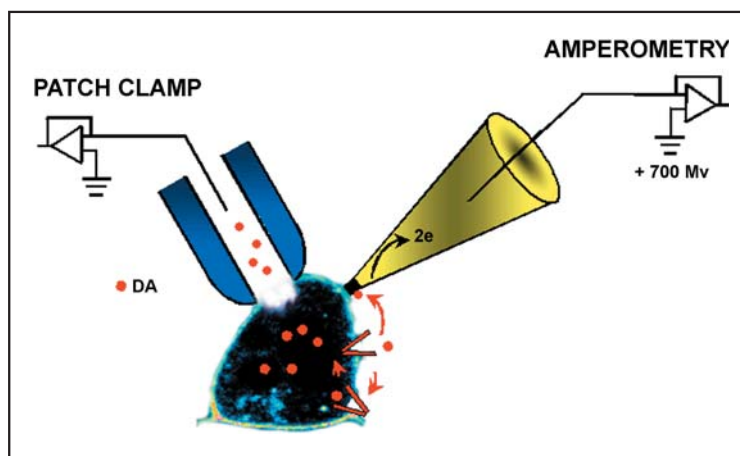
60 amino acids. Many of these amino acids are potential sites of phosphorylation, a chemical reaction in which a phosphate group is added through the action of enzymes called kinases. Amphetamine has been shown to increase kinase activity and Margaret Gnegy, a coauthor of the current research article, showed previously that inhibiting protein kinase C activity blocks amphetamine’s ability to release dopamine. Therefore, Javitch, Galli, and Gnegy hypothesized that N-terminal phosphorylation of DAT might play a critical role in the dopamine overload caused by amphetamine.

The researchers found that amphetamine-induced dopamine release was reduced by 80% in cells expressing a mutant dopamine transporter in which the first 22 amino acids of the

N-terminal domain had been removed (del-22). Surprisingly, this truncated transporter displayed normal dopamine uptake. In a full-length DAT, mutation of the five N-terminal serine amino acids to alanine amino acids, which prevented phosphorylation, produced an effect similar to removing the 22 amino acids. In contrast, replacing these five serine residues with aspartate residues to mimic phosphorylation

led to normal dopamine release as well as normal dopamine uptake.

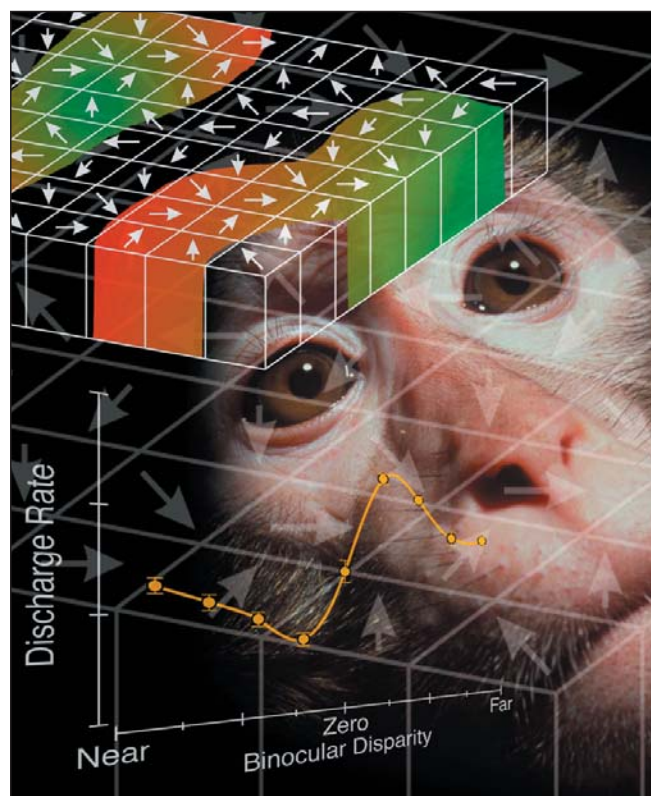
These findings suggest that phosphorylation of one or more of these serine residues is necessary for amphetamine to flood the synapses with dopamine. While phosphorylation is a normal mechanism for regulating protein activity in a cell—and DAT is “significantly phosphorylated” under normal conditions—amphetamine could increase the level of DAT phosphorylation. Elucidating the mechanisms through which phosphorylation of DAT’s N-terminus facilitates dopamine overload could lead to the development of drugs that block the “rewarding” effects of amphetamines and other addictive psychostimulants without interfering with normal dopamine clearance.



Measuring changes in dopamine transport

Khoshbouei H, Sen N, Guptaroy B, Johnson L, Lund D, et al. (2004) N-terminal phosphorylation of the dopamine transporter is required for amphetamine-induced efflux. DOI: 10.1371/journal.pbio.0020078

Microstimulation of Neurons Distinguishes Neural Contribution to Perception



The neural contribution to monkeys' perception of motion

The brain is an overwhelmingly complex organ packed with billions of nerve cells, performing a myriad of different functions. To decipher the roles of individual neurons in processing sensation or actions, scientists can measure the neural activity of animals that are shown particular objects or perform simple tasks. In this way, neurons are categorized as having preferences, also known as selective responses. These techniques have been particularly helpful in determining, or mapping, preferences of visual areas in the cerebral cortex. For example, some neurons respond to the color of an object, while others respond to the direction that object is moving. What is less well understood, however, is how the brain integrates information from individual neurons for complex processes such as perception and behavior. That is, how does neural activity affect what we see and do?

Microstimulation, a technique that activates a cluster of nerve cells by zapping them with a weak electrical current, has helped make causal links between neurons and behavior. For instance, when neurons in an area of the visual cortex that are “tuned” to a particular direction of motion are microstimulated, the way monkeys perceive moving dots on a video screen changes. Microstimulation seems to change what they see. Similar work has also been done for neurons that respond to binocular disparity—the depth-of-field information you gain because each eye has a slightly different view of the world.

But many neurons respond, or are tuned, to more than one dimension, leading scientists to wonder how information from these multidimensional neurons contributes to perception—especially when some of that information is irrelevant to a given task. As they report in this issue, Gregory DeAngelis and

William Newsome find that neurons tuned to both direction and binocular disparity contribute little to monkeys' perception of motion.

The researchers asked three rhesus monkeys to determine the direction a group of dots was moving on a TV screen—a task that can be done regardless of the perceived depth of the dots. The authors had already located two different types of neurons in each of the monkey's brains: sites tuned strongly to direction and multidimensional sites tuned to both direction and binocular disparity. They then determined each site's exact preference: the direction of motion and degree of binocular disparity (if present) that triggered maximum neural activity. The researchers then showed the monkeys several sets of video displays, some with the dots moving in the “preferred” direction and some not. The microstimulation acts somewhat like adding dots in the preferred direction—which confuses monkeys when the real dots are moving against preference and aids them in trials of preferred moving dots. If the behavioral effect of microstimulation—whether it be a help or a hindrance—was significant, it meant that the monkeys were monitoring the activated neurons to perform the task at hand; if there was no change, the stimulated neurons were not being recruited.

DeAngelis and Newsome hypothesized that multidimensional neurons (which are also tuned to the irrelevant dimension of binocular disparity) might be ignored during pure motion perception tasks. For two of the three monkeys, this was true. Microstimulation of multidimensional sites had no effect on their behavior, compared to the significant effect of microstimulation of direction-only sites. But for the third monkey, called monkey R, microstimulation of both types of sites had significant effects on his performance. He didn't seem to be ignoring anything. The authors proposed that the monkeys could be using different neural strategies to complete the same task. This conclusion is supported by the fact that monkey R performed better on the task than the other monkeys; he appeared to be recruiting any neuron with applicable information, unlike the others, who seemed to rely on neurons tuned solely to direction of motion. Furthermore, for the few multidimensional sites that affected behavior, their contribution was tempered by how well the depth, or disparity, of the video matched the preference of the stimulated neurons.

The results of this paper show that even if neurons carry information that can aid in perceptual decision making, they may not participate, depending on how they are tuned along other (irrelevant) stimulus dimensions. All directional neurons are not created equal—some are more useful than others for a particular task. Whether neurons that respond to a particular stimulus contribute to the task at hand depends on how closely that stimulus hews to the neurons' preference as well as on the subject's learned strategy for performing the task. This neural flexibility, the authors point out, suggests that the brain uses complex, variable strategies to respond to changing environmental stimuli. Techniques like microstimulation will be helpful in drawing the connections between neural activity and behavior.

DeAngelis GC, Newsome WT (2004) Perceptual “read-out” of conjoined direction and disparity maps in extrastriate area MT. DOI: 10.1371/journal.pbio.0020077