

Synopsis of Research Articles

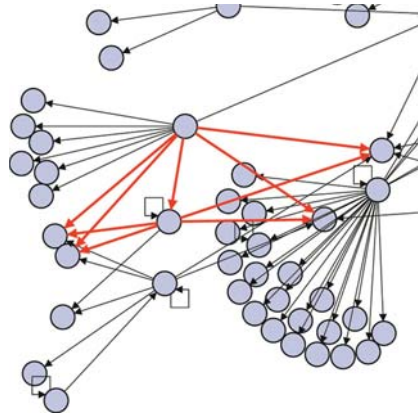
Charting the Interplay between Structure and Dynamics in Complex Networks

DOI: 10.1371/journal.pbio.0030369

While intelligent-design proponents enjoy their 15 minutes of fame denying the role of evolutionary forces in generating complex networks in nature, scientists are probing the organizing principles that govern these networks. Traditional models of complex networks assumed that connections between units—such as genes, proteins, neurons, or species—occur randomly. These notions changed as studies of protein interaction networks and other biological systems revealed “small world” features—characterized by short paths between nodes and highly clustered connections—and varying levels of organization, with certain patterns of local connections occurring more frequently in complex networks than in random networks. What determines the abundance of these so-called network motifs in specific networks is not known.

To study complex, dynamic systems, researchers create graphical representations with network maps. Though the network structure is static, the nodes represent values that change with time. For example, the neural map of the worm *Caenorhabditis elegans* contains 302 neurons (represented as nodes) and roughly 7,000 synaptic connections that appear fixed even though they represent transient behavior, such as activation states of individual neurons and their probable interactions. Discerning the global dynamics of these network structures has proved a major challenge.

In a new study, Robert Prill, Pablo Iglesias, and Andre Levchenko use the power of computational analysis to tackle the problem of identifying the dynamic features of a large-scale network. They found a high correlation between a dynamic property of a network motif—ability to withstand small fluctuations in the system—and its relative abundance in well-characterized biological networks. Their results suggest that just as connections between individual components of a biological network—be they genes, proteins, or cells—influence function, the dynamic properties of a network motif relate to the motif’s function and could determine its prevalence in biological networks.



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In this schematic of a transcriptional network, nodes represent operons (a set of bacterial structural genes and their regulatory elements) and links represent transcription factor DNA binding interactions. (Image generated with Cytoscape v1.1.)

For a network motif to qualify as stable, it must return to steady state after small-scale perturbations, defined as intrinsic random fluctuations, or noise, and transient oscillations in activity. The behavior of a motif is determined by the direction, sign (presence of positive or negative feedback loops), and strength of the connections. The authors varied these parameters to simulate motif response to small perturbations. To measure stability, the authors assigned a structural stability score (SSS) as the probability that a particular motif returns to a postperturbation steady state. They use this metric to analyze the dynamics of all possible three- or four-node networks (noting that even two-node networks exhibit complex behavior). Based on the SSS scores, all the structurally distinct three- and four-node network motifs fell into three distinct categories: robustly stable circuits with no feedback loops, moderately stable circuits with a single two-node feedback loop (assuming a negative feedback loop), and, least stable, a mixture of complicated, highly connected motifs.

Comparing motif abundance in known biological networks with the SSS scores of simulated motifs revealed

an “excellent correlation” between stability and motif abundance. Higher stability motifs were more abundant in the real networks, while low-stability motifs were absent, suggesting that the nonrandom character of network organization is driven by the structural stability of network motifs. To see how these motifs might operate as functional units, the authors used microarray data from yeast subjected to five different environmental stresses, including mild heat shock and hydrogen peroxide treatment, and mapped activated genes (represented as nodes) to their locations in the network. All the active regulatory motifs had a high stability score, suggesting that the nonrandom nature of the yeast transcriptional network may have arisen from selection acting on small motifs that respond robustly to specific environmental stresses. Expanding their analysis to other biological networks, the authors found that yeast and the pathogen *Escherichia coli* have similar motif profiles, likely reflecting similar environmental pressures, while the fruit fly transcription program and worm neuron network contain different motifs, reflecting both different environmental and functional demands.

These results suggest that both global constraints on the network and properties of network motifs themselves influence the abundance of motifs and the overall structure of a given network. While the authors caution that their networks are stripped-down versions of those found in biological systems, they point out that their approach can incorporate more complicated interactions as understanding of living networks increases. And with this new understanding, scientists can test the hypothesis that selective pressures favor motifs with particular dynamic properties. For more information on structural stability and networks, please see the accompanying Primer by Doyle (DOI: 10.1371/journal.pbio.0030392).

—Liza Gross

Prill RJ, Iglesias PA, Levchenko A (2005) Dynamic properties of network motifs contribute to biological network organization. DOI: 10.1371/journal.pbio.0030343

Alternative mRNA Splicing: Control by Combination

DOI: 10.1371/journal.pbio.0030406

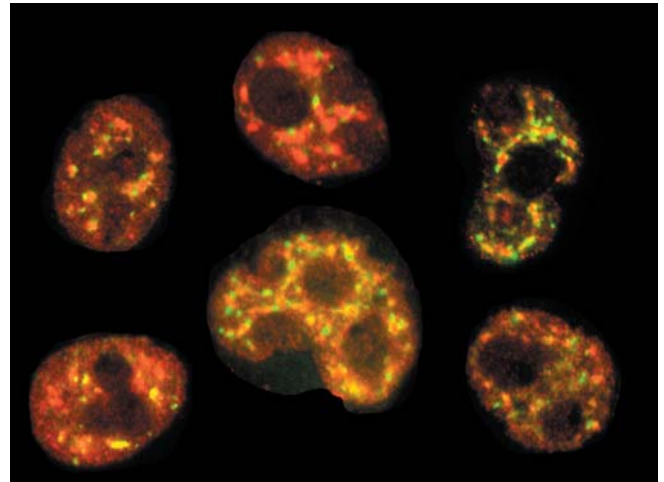
In 1977, a flurry of papers ushered in a radical new concept in molecular biology—the idea of RNA splicing. It had been known for some years that the information for building organisms is stored as DNA sequences, which are transcribed into messenger RNAs (mRNAs) before translation into proteins. Although it had been established that the DNA and mRNA sequences line up exactly in bacteria, molecular biologists began to suspect in the mid-1970s that the genomes of eukaryotes (organisms with nuclei) are organized somewhat differently. Eukaryotic genes, it turns out, are encoded in small sections scattered over enormous distances of DNA. To make proteins from these “split genes,” the whole length of DNA is transcribed into pre-mRNA and then converted into mRNA by spliceosomes—molecular machines that remove the non-coding pieces of RNA (the introns) and splice together the protein-coding pieces (the exons).

One important consequence of RNA splicing is that one gene can produce several different mRNA variations, or isoforms, simply by stitching together different combinations of exons. For example, a single gene in vertebrates encodes calcitonin (a thyroid hormone that controls calcium levels) and calcitonin-gene-related peptide (a neuropeptide). Alternative splicing also contributes to human disease—for instance, the selection of different splice sites generates aberrant ratios of mRNA isoforms in several neurological diseases.

But how are these alternative splice sites selected? One popular model proposes that alternative splicing in mammalian cells is largely controlled by binding of general splicing factors to pre-mRNA molecules during the formation of the spliceosome. The spliceosome contains many of these factors, including a class of proteins called SR proteins, which contain one or two RNA-binding domains and a protein–protein interaction domain that is rich in serine and arginine amino acids. An important prediction of the combinatorial model for control of alternative splicing is that alternatively spliced transcripts will recruit different combinations of pre-mRNA splicing factors *in vivo*. New data from Mabon and Misteli support this prediction.

Pre-mRNA splicing factors accumulate at sites of active transcription, and splicing and can be detected and quantified in individual living cells by tagging the splicing factors with fluorescently labeled antibodies. So, to see whether different factors accumulate at alternatively spliced transcripts, the researchers developed stable cell lines carrying versions of the gene encoding a protein called tau designed to splice in different ways. In healthy people, exon 10 of the *tau* gene is included or excluded from *tau* mRNA with roughly equal probability during pre-mRNA splicing; in people with a rare Parkinsonism-like neurological disorder, mutations near one end of exon 10 result in its predominant inclusion. The researchers, therefore, examined cell lines carrying *tau* genes with and without a mutation of this type to change the ratios of mRNA transcripts including or excluding exon 10. Their results show that a subset of SR protein splicing factors is efficiently recruited to *tau* transcription sites that produce exon 10-containing mRNA, but less efficiently recruited to transcription sites where exon 10 is excluded.

These results provide the first *in vivo* evidence for the differential association of pre-mRNA splicing factors with alternatively spliced transcripts, and support a combinatorial mechanism for spliceosome formation. Exactly which splicing



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Pre-mRNA splicing factors (red) are recruited to sites of transcription and splicing (green) in the nucleus of mammalian cells.

factors are recruited to each spliceosome would depend on both the concentration of each factor in individual cell types and the regulatory elements present in each pre-mRNA. The combinatorial mechanism for the control of alternative splicing, the authors suggest, could allow cells to adjust splicing outcome (and consequently which proteins they express) rapidly in response to intracellular or extracellular cues, as well as contributing to the generation of protein diversity. —*Jane Bradbury*

Mabon SA, Misteli T (2005) Differential recruitment of pre-mRNA splicing factors to alternatively spliced transcripts *in vivo*. DOI: 10.1371/journal.pbio.0030374

Wild Gorillas Handy with a Stick

DOI: 10.1371/journal.pbio.0030385

When chimpanzees were first seen using tools in Liberia in 1951, little was known about great ape behavior in the wild. The sighting was published as a short note by Harry Beatty in the *Journal of Mammalogy* (recounted by primatologist Frans de Waal in *The Ape and the Sushi Master*). After spotting a shell mound, Beatty saw a chimp “come ambling round a bend bearing an armful of dried palm nuts,” sit down beside a rock, select a nut, place it on a flat rock surface, and pound it with another rock to extract the meat. Thirty years passed before primatologists would describe the same behavior in chimps in Guinea. By then it was clear that nonhuman primates used objects for many tasks, from intimidating rivals and predators to taking leaf sponge baths and processing food. Still, the notion that tool use was not the sole province of human intelligence was difficult for some to accept.

Though chimps, bonobos, orangutans, and gorillas all use tools in captivity, often imitating behaviors of their keepers, tool use had not been observed for gorillas in the wild. (Chimps famously use sticks to fish for termites and rocks to crack nuts in the wild; orangutans use sticks to extract seeds from the spiny, sharp husk of *Neesia* fruits in the wild, and bonobos use



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A first time for everything: This adult female gorilla in Nouabalé-Ndoki National Park, northern Congo, uses a branch as a walking stick to gauge the water's depth, proving that gorillas use tools too.

various tools, including moss as sponges and leafy twigs to swat away bees.) That no one has ever observed wild gorillas using tools—despite decades of intensive study—prompted scientists to speculate that gorillas lost the skill because they didn't need it. The largest of the great apes, gorillas can gnash nuts between their teeth and simply smash termite mounds to release their denizens.

But now, as part of an ongoing study of western gorillas in Nouabalé-Ndoki National Park in the Republic of Congo, Thomas Breuer, Mireille Ndoundou-Hockemba, and Vicki Fishlock reveal that gorillas are just as resourceful as the other great apes. From an observation platform at Mbeli Bai, a swampy forest clearing

that gorillas frequently visit to forage, Breuer et al. observed an adult female gorilla named Leah (a member of a long-studied gorilla group) at the edge of a pool of water, "looking intently at the water in front of her." Leah walked upright into the water, but stopped and returned to the edge when the water reached her waist. She then walked back into the water, grabbed a branch in front of her, detached it, and, grasping it firmly, repeatedly jabbed the water in front of her with the end of the branch, "apparently using it to test the water depth or substrate stability." She continued walking across the pool, branch in hand, "using it as a walking stick for postural support."

In a separate incident, a second adult female named Efi (who belonged to a different group) emerged from the forest and broke off the thin, long trunk from a dead shrub with both hands. She then jammed the trunk into the ground and grasped it with her left hand while manipulating food with her right hand. Efi then placed the trunk on the swampy ground in front of her, and, walking upright, used it as a bridge to cross the muck.

That tool use was seen in lowland gorillas and not mountain gorillas—the most intensively studied gorillas—may reflect the different ecological challenges facing the two populations, supporting the notion that ecology influences the evolution of novel tool use. Though Mbeli Bai gorillas often use intact branches to help them get around, the authors explain, these are the first observations of wild gorillas using detached objects as tools. However the gorillas acquired their skills, these observations suggest that the intelligence required for tool use evolved before the gorilla lineage split off from humans and the other great apes—providing further evidence that intelligence is not unique to humans. —*Liza Gross*

Breuer T, Ndoundou-Hockemba M, Fishlock V (2005) First observation of tool use in wild gorillas. DOI: 10.1371/journal.pbio.0030380

Experimentally Validated Model Accounts for T Cells' Discriminating Ways

DOI: 10.1371/journal.pbio.0030391

When a pathogen slips into the body, it might infect a cell or get eaten by a specialized white blood cell. Either way, its proteins get chopped into peptide fragments, loaded onto molecules encoded by genes in the major histocompatibility complex (MHC), and sent to the cell surface as a peptide-loaded MHC molecule (pMHC) for immune surveillance. The immune system can rally against billions of pathogens, in part because every T cell expresses a unique receptor (TCR), acquired during development, that recognizes specific pMHCs. Developing T cells undergo a selection process that weeds out more than 98% of cells, leaving only those cells whose receptors react to "self" pMHCs enough to signal but not enough to fully activate the T cells. Because antigen-presenting cells (APCs) in an infected host bear both self and pathogen-derived pMHCs, proper immune function depends on the discriminatory capacity

of TCRs: they must allow a full T cell response to foreign antigens and avoid reacting to self-peptides on the same cell surface.

When a T cell homes in on a pathogenic antigen, the response is quick, sensitive (just a few molecules can set them off), and digital (all or nothing), engaging signaling pathways necessary for T cell activation, proliferation, and survival. (Molecules called agonist ligands trigger TCR signaling.) Signals relay through the cell as kinase enzymes activate proteins by triggering chemical reactions that add one or more phosphates (a process called phosphorylation); removing phosphates (called dephosphorylation) deactivates the proteins and the signal decays.

One explanation for this rapid, sensitive response is that pMHCs induce unique conformational changes in the receptor to initiate signaling. But a TCR can react differently to the same pMHCs at distinct

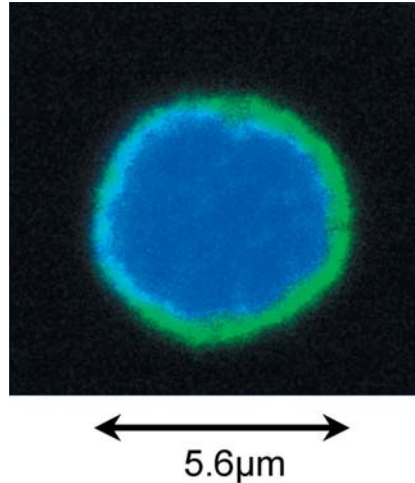
points in its life history, and binding pairs show no conformations specific to agonists versus non-agonists. Alternately, in the kinetic proofreading concept, a kinetic threshold related to the TCR–pMHC binding properties accounts for ligand discrimination—though in simple forms of this hypothesis discrimination occurs at the expense of a rapid, sensitive, digital response.

Hoping to resolve these discrepancies, Grégoire Altan-Bonnet and Ronald Germain combined computer modeling with experimental results to develop a quantitative model of early TCR signaling. The authors revealed a novel aspect of TCR signaling—the explosive digital response of a key enzyme in the pathway—and identified competing feedback systems that may explain how T cells combine selectivity with a rapid, sensitive response.

To construct a predictive model, Altan-Bonnet and Germain measured

kinetic aspects of biochemical responses to TCR–pMHC pairing by focusing on extracellular signal-related kinase (ERK), a key player in the TCR signaling cascade. Using T cells harvested from transgenic mice engineered to produce identical TCRs and APCs engineered to express very few self-pMHCs, the authors studied the conditions required for ERK activation. In individual T cells, the ERK pathway was activated by as few as ten foreign ligands. These experiments also revealed a “previously unappreciated aspect” of ERK signaling in T cells: it either occurred or it didn’t, but when it did, the result was 100,000 phosphorylated ERK enzymes.

T cells that showed this explosive response could also distinguish between pMHCs with minor differences in TCR binding time, reinforcing the importance of the SHP-1 negative feedback system in preventing “sneak-through activation” by non-agonists. (SHP-1 is a dephosphorylating enzyme.) To test this hypothesis in a quantitative manner, Altan-Bonnet and Germain constructed a TCR signaling model in which kinetic proofreading of TCR–ligand interactions produces a quick initial negative feedback response (mediated by SHP-1) and a delayed, explosive digital ERK positive feedback system that, once activated, overrides the negative pathway and allows productive signaling. The authors then ran signaling simulations



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Confocal microscopy was used to determine the cytoplasmic volume of T cells to infer concentrations of signaling molecules in the cytoplasm.

using different ligand numbers with different TCR binding lifetimes. Their model “shows almost absolute discrimination” between closely related pMHCs while preserving a fast, sensitive response to just a few agonist ligands.

The model also yielded predictions that the authors validated experimentally: the ERK response slows down dramatically at low ligand densities; negative feedback adjusts to ligand strength and quantity to prevent

signaling by high concentrations of low-affinity ligands, and allows sensitive responses to low concentrations of high-affinity ligands; differential activation of the negative feedback explains the existence and hierarchy of antagonism in T cell activation; and mature differentiating T cells permit signaling with different levels of ligand discrimination, depending on intracellular concentrations of molecules such as SHP-1.

Altogether, these results suggest that ligand discrimination is not “hard-wired” into TCR–ligand structural affinities. Rather, the threshold that permits TCR signaling varies as concentrations and dynamics of intracellular molecules vary during T cell development and after antigen activation. The model described here, though a necessarily simplified version of TCR signaling, highlights the effectiveness of simple feedback loops in helping cells filter out unwanted signals while effecting quick, sensitive responses—properties crucial for most other regulatory networks. The model also reinforces the importance of detailed probing of cell signaling dynamics to better understand the functions of a living system. —*Liza Gross*

Altan-Bonnet G, Germain RN(2005) Modeling T cell antigen discrimination based on feedback control of digital ERK responses. DOI: 10.1371/journal.pbio.0030356

Molecular Signatures of the Developing Hair Follicle

DOI: 10.1371/journal.pbio.0030366

When a human baby enters the world, some 5 million hair follicles cover its body. For many, these follicles cease production with age, a fate that befalls both men and women, accounting for a multibillion-dollar hair growth industry, despite the products’ limited success. Though the stages of hair development are understood, the molecular signals that guide the process are less clear.

Hair follicles develop from cells in the ectoderm, an embryonic epithelial layer that gives rise to the surface epidermis, and the underlying mesenchyme, connective tissue cells derived from the embryonic mesoderm. During fetal development, the spatial position and individual characteristics of the follicles (long versus short, for example) are determined. Once established, normal hair growth continues in cycles, with each follicle passing through three distinct stages before the hair is shed and the cycle begins anew. Both embryonic follicle formation and adult hair growth begin when mesenchymal dermal papilla (DP) cells, clustered beneath a single layer of epidermal cells, send the message to epithelial stem cells to make a hair follicle. Epithelial stem cells then send their progeny, epithelial matrix cells, to surround the DP cells and trigger the sequence of events that culminates in new hair.

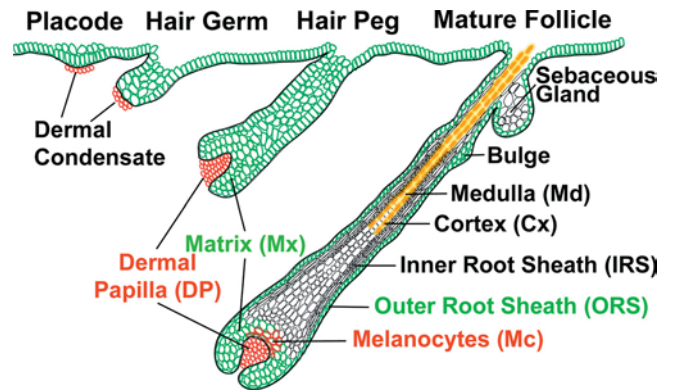
To shed light on the molecular program that drives these mesenchymal–epithelial interactions, Michael Rendl, Lisa Lewis, and Elaine Fuchs used a cell-sorting technique that allowed them to isolate pure populations of DP cells along with populations of four neighboring cell types. Using microarrays to analyze the gene expression profiles of the different cell populations, the authors identified molecular signatures for the DP and its niche, including a group of little-studied genes linked to hair disorders. With these molecular signatures, researchers can begin to analyze each gene’s role in hair development and growth.

To get the purified cell populations for their studies, the researchers used an innovative combination of existing techniques, and took advantage of the underlying biology of the hair follicle. In mice, hair begins to grow on the animal’s back around 15 days in utero, and is fully formed roughly four days after birth. After the matrix cells envelop the DP, they proliferate, migrate upward, and differentiate into the hair shaft and the inner root sheath (IRS), which surrounds the hair. The IRS is covered by an outer root shaft (ORS), and the whole structure is enclosed by a membrane that separates the skin epithelium from the dermis and the DP. (Melanocytes, which determine hair color, sit just above this membrane.)

To extract cells from the DP along with its neighboring cells, Rendl et al. used transgenic mice and a cell-sorting technique called fluorescent-activated cell sorting, so they could retrieve the different cell types from the backs of the mice. Mice expressing red fluorescent protein (RFP) were bred with mice expressing green fluorescent protein (GFP). Because both reporters express the fluorescent proteins under known conditions—the promoter used to control RFP is typically expressed in DP cells, and the promoter used to control GFP is expressed in matrix and ORS cells—they serve as an initial filter in cell sorting.

Follicles were isolated from the backs of four-day-old mice, and five populations of cells—matrix, ORS, melanocytes, DP, or dermal fibroblasts (connective tissue cells)—were sorted based on whether or not they expressed GFP or RFP and whether they expressed known cell-surface markers. The authors were most interested in identifying the unique features of the cells that initiate the hair development program, the DP cells. They were sure the cells were from the DP because they expressed high levels of four known DP markers and triggered hair growth on mutant *Nude* mice, which only DP cells can do (along with ubiquitous keratinocytes).

Microarray analysis of the gene activity of the different cell types found that 4,000 genes were common to all the cell types, forming the “molecular backbone” of basic cellular functions. Just 150–300 genes showed elevated expression in any one cell type, forming the molecular signature for each population. Because the authors’ DP signature genes differed substantially from those found in previous studies, they went on to verify protein expression in the cells, confirming that the signature accurately reflected the DP profile. Many of the signature genes are involved in genetic hair disorders, confirming the functional significance of the signatures, while others encode novel proteins involved in transcription, cell signaling, and cell



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Having identified the molecular signatures of the dermal papilla and its neighboring cells, researchers can now study how these genes influence hair development and growth.

adhesion. This subset provides a window into the special roles carried out by the different cells at the genomic level, and will help future studies explore their function in hair biology.

Developmental biologists have made great progress in understanding the molecular agents of hair morphogenesis since Margaret Hardy called the hair follicle a “treasure waiting to be discovered” just over a decade ago. By identifying unique molecular profiles of the DP and its cohorts, this study has paved the way for revealing all the hair follicle’s secrets, one signal at a time. For more on skin and hair follicle development, see the accompanying Primer by Sarah Millar (DOI: 10.1371/journal.pbio.0030372). —*Liza Gross*

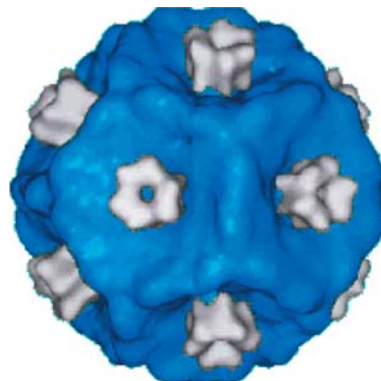
Michael Rendl, Lisa Lewis, Elaine Fuchs (2005) Molecular dissection of mesenchymal–epithelial interactions in the hair follicle. DOI: 10.1371/journal.pbio.0030331

A Role for Selection in the Evolution of Genetic Robustness

DOI: 10.1371/journal.pbio.0030407

When Darwin proposed the interplay between variation and natural selection as the driving force of evolution, he had no idea what material produced that variation. Ninety-one years later, Hershey and Chase’s famous blender experiment identified the source of variation as DNA. Today, biologists are still struggling to elucidate the details of that interplay.

Natural selection works on genetic variations that produce physical changes in an organism. Think of an organism as a collection of genes (its genotype), and its physical characteristics (its phenotype) as its genotype interfacing with the environment, a natural laboratory that saves or discards a genotype based on the performance of its phenotype. Assuming that a population of genotypes is well adapted to its environment, most mutations are likely to reduce performance in that environment. Thus, populations at equilibrium should experience selection for mechanisms that



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RNA phage viruses that co-infect host cells with more fit viruses can withstand high mutation loads with the help of their fitter counterparts—but only for so long.

protect the phenotype against mounting deleterious mutations—a phenomenon called mutational (or genetic) robustness. Most of the evidence that robustness

arises from selection rather than chance comes from theoretical studies and from studies of “digital organisms”—computer programs that self-replicate, mutate, and evolve—and has proved difficult to establish in the lab.

In a new study, Rebecca Montville, Paul Turner, and their colleagues provide experimental evidence for adaptive genetic robustness by working with a mutation-prone virus that infects bacteria, called RNA phage $\phi 6$. (Viruses that infect bacteria are called phages.) Though theoretical predictions for mutational robustness assume that phenotype expression results solely from the underlying genotype, many viruses can overcome their own mutational deficiencies by co-opting the proteins produced by more fit viruses co-infecting the same host, a feature called complementation.

In a previous study, the authors demonstrated for RNA phage $\phi 6$

that complementation buffers less fit viruses against the harmful effects of mutations. They then created six replicate populations of phages and allowed them to adapt to their bacterial host over hundreds of virus generations; three populations evolved at a low ratio of infecting viruses to bacteria (called low multiplicity of infection [MOI]) and three at a high MOI. Populations at high MOI experienced higher rates of co-infection. In this study, the authors investigated the evolutionary consequences of this phenomenon with the hypothesis that selection for mutational robustness should be relaxed for co-infecting phages, since phenotype constancy is bolstered by co-infection with their fitter viral companions.

Using clones from their six replicate populations, Montville et al. generated 60 new lineages and subjected them to a mutation accumulation

experiment under conditions that allowed mutations to accumulate at roughly the same probability in high and low MOI lineages. The authors then evaluated the fitness consequences of mutation accumulation on the lineages by comparing their growth rate in the bacteria before and after mutation accumulation. The authors found greater variance in fitness change for the high co-infection lineages compared to the low-infection lineages, supporting the hypothesis that selection for mutational robustness is stronger in the absence of co-infection.

The authors go on to rule out the notion that different fitness effects were produced by different mutation rates in the lineages. Interestingly, the less robust viral genomes were copied more accurately than their more robust counterparts; why less accurate genome replication might accompany the

evolution of robustness is a question for future study.

While complementation appears to buffer the damage of mutational onslaughts in the short-term, this benefit of co-infection eventually disappears because the buffer slows the rate that harmful mutations are culled from the virus population. The authors highlight an additional cost of co-infection: by weakening selection for robustness, co-infection may favor the evolution of genomes that are more vulnerable to the harmful effects of mutation. Future work is needed to examine this seeming tug-of-war between short-term and long-term consequences of co-infection, and its impact on the evolution of virus traits.

—*Liza Gross*

Montville R, Froissart R, Remold SK, Tenaillon O, Turner PE (2005) Evolution of mutational robustness in an RNA virus. DOI: 10.1371/journal.pbio.0030381

Geographic Spread of an AIDS-Resistant Mutation

DOI: 10.1371/journal.pbio.0030397

The discovery in the 1990s of a gene variant that thwarts HIV infection triggered development of a promising new class of medications. The gene normally encodes a protein receptor, called CCR5, that sits on the surface of white blood cells. HIV gains entry to these cells through CCR5. The variant gene, or allele, contains a mutation—called $\Delta 32$, because 32 base pairs are deleted—that produces truncated CCR5 receptors that are useless to the virus, conferring resistance to individuals with both copies of the mutation, and delaying disease progression to those with one copy. The $\Delta 32$ mutation also raised interesting questions for evolutionary biologists.

About 10% of Europeans and inhabitants of western Asia carry the mutation, which researchers think evolved at least 700 years ago—yet HIV emerged only about 50 years ago. According to population genetics theory, for a mutation to be neutral, or confer no selective advantage, it would have to be much older to occur at such a high frequency in the population. This inconsistency raised the possibility that the mutation spread because it provided an advantage against some other selective factor, now thought to be smallpox.

The spread of advantageous alleles within a population is a fundamental aspect of evolution. While theoretical models have studied the dynamics of dispersal, few studies have tracked real-life examples or developed statistical methods to investigate the process. Now, John Novembre, Alison Galvani, and Montgomery Slatkin use the geographic distribution of the $\Delta 32$ mutation as an example of the process, and estimate the effects of selection relative to dispersal on $\Delta 32$ to shed light on its origins and spread through Europe. Their model shows that, given its age, the mutation spread rapidly due to long-range dispersal and intense selection to attain its current range.

The mutation follows a north-south spatial gradient, from northern Europe to Greece. Since the broadest area of high frequency is northeastern Europe—based on the population genetics assumption that a mutation originated where it is



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This contour map shows the European distribution of the CCR5 $\Delta 32$ variant, which was used to develop a method for studying the geographic spread of gene variants under positive selection.

most abundant—one hypothesis is a Viking origin. The Viking hypothesis, first put forth in the 1990s, proposes that the allele was present in Scandinavia at least 1,000–1,200 years ago, and Vikings carried it north to Iceland, east to Russia, and south to central and southern Europe. Alternately, the mutation may have arisen in central Europe and then increased in frequency in the

north (if selection pressures there increased the advantage of having the allele), or it may have arisen in the north and spread as individuals (not Vikings) migrated to other areas.

To quantitatively test these competing hypotheses, Novembre et al. used allele frequencies collected from 71 locations and simulated the spread of the mutation by varying the origin of the mutation, the relative strength of selection to dispersal, and the strength of gradients in selection. The authors then calculated the probability of the allele frequency data given the parameters, and then found the parameter set that best fit the data. The results indicate that, given its age, strong selection and long-range dispersal (perhaps through trade routes or migrations) are the most likely explanation for the present distribution of the $\Delta 32$ mutation. The data show a north–south gradient in allele frequency, supporting a modest north–south gradient in selection intensity. As for the mutation’s provenance, a northern European origin is supported only by assuming uniform selection; assuming a selection gradient points to a more southern origin, perhaps in Spain, or in northern Germany (when data from Iceland are included).

Altogether, the results suggest caution regarding the Viking hypothesis: though the long-range dispersal findings are consistent with the hypothesis, the authors conclude, a southern origin “raises the possibility that the allele arose outside of Scandinavia and spread into the region via dispersers from the

south.” The results also show that modest selection gradients—where the intensity of selection differs slightly between distant locations—allow for an allele to originate far from its current abundance.

By applying a standard model of the spatial spread of an advantageous allele to a map of Europe and west Asia and adding a statistical component to the model, Novembre et al. could analyze the allele frequency data from these two regions. Though a new study by Pardis Sabeti et al. (DOI: 10.1371/journal.pbio.0030378), also in this issue, presents evidence that CCR5 $\Delta 32$ is older than previously estimated and that its frequency could be explained by neutral selection, the findings don’t discount the validity of the model presented here. In fact, if selection on CCR5 $\Delta 32$ were weaker, then the dispersal estimated in this model would become weaker—falling more in line with historical estimates. However the mutation arose, this approach will allow researchers to tease apart the relative contributions of dispersal patterns and local selection pressures to study the geographic distribution and evolutionary history of any mutation—critical tools for tracing the relatively recent evolutionary history of humans. —*Liza Gross*

Novembre J, Galvani AP, Slatkin M (2005) The geographic spread of the CCR5 $\Delta 32$ HIV-resistance allele. DOI: 10.1371/journal.pbio.0030339

Charting the Path of the Deadly Ebola Virus in Central Africa

DOI: 10.1371/journal.pbio.0030403

Thanks to sensationalized accounts of patients with liquefying flesh and spouting blood, the Ebola virus may well be the most feared disease on the planet. But the reality of the virus, which strikes humans and other primates, is grim enough, with patients experiencing sudden onset of fever, headache, intense weakness, and muscle pain, followed by diarrhea, vomiting, severe rash, organ failure, and massive hemorrhaging, sometimes external, within two to 21 days of exposure. The first human Ebola outbreaks occurred between 1976 and 1979 in Sudan and Zaire (now the Democratic Republic of Congo), where 88% of the 318 infected persons died—a typical mortality rate for this strain, called the Zaire strain of Ebola virus (ZEBOV). It’s thought that humans acquired the virus after handling infected gorilla and chimp carcasses.

Over the past ten years, separate outbreaks of the deadly Zaire strain have killed hundreds of humans and tens of thousands of great apes in Gabon and the Republic of Congo—which harbor roughly 80% of the last remaining wild gorilla and chimpanzee populations. Between 1983 and 2000, poaching and logging precipitated catastrophic declines in these great apes, but scientists fear that Ebola may pose an equally



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Repeated outbreaks of the Zaire strain of the Ebola virus (Ebola virions pictured above) in central Africa were caused by a recent spread of the virus, rather than by a long-persistent strain at each site.

deadly threat. Any efforts to contain the next epidemic depend on understanding the dynamics of the virus’s spread.

In a new study, Peter Walsh, Roman Biek, and Leslie Real combined genetic data with information on the timing and location of past ZEBOV outbreaks to determine the merits of two competing hypotheses to explain the emergence and spread of the virus. In the prevailing view, ZEBOV arose from long-persistent local strains after increased contact between humans or great apes and an

unidentified reservoir host. But Walsh et al. found support for the alternative hypothesis: that ZEBOV had recently spread to the outbreak regions. This is good news because a virus that spreads at a predictable rate in a predictable direction is far easier to control than one that emerges by chance or at the hands of an unknown trigger.

The authors modeled the virus’s spread based on assumptions of a long-persistent virus versus a recently emerged virus, and tested the predictions of these competing hypotheses using genetic data—gathered from gene sequences taken from human samples at the different outbreak sites—and information on the spatiotemporal dynamics of the outbreaks. Charting the evolutionary relationships of the viral genotypes identified one major lineage with a most recent common ancestor consistent with the 1976 outbreak. Comparing the path of descent with outbreak localities mirrored the timing of the outbreaks, with new outbreaks directly descending from those preceding.

Analyzing the spatiotemporal pattern of outbreaks revealed a spread at the rate of about 50 kilometers/year—a predictable path not likely for a persistent virus—with the first

epidemic in Yambuku, then spreading south to Kikwit and west to Bououé, Gabon. Plotting the geographic distribution of genotypes revealed a clear spatial structure at both local and regional scales: the genotypes from the 2001–2003 Gabon/Congo outbreaks, for example, decreased in genetic similarity as distance increased. Again, this finding is consistent with the recently emerged hypothesis, which predicts a correlation between genotype and geography at different distances. Simulations of viral evolution in a spreading epidemic also showed a consistent spread pattern and a strong correlation between genetic divergence and spatial separation.

Though the authors can't say how the virus was transmitted, the simulations showed that a "simple nearest neighbor contact process" could produce the linear, uniform spread rates found here.

Though the strength of the individual lines of evidence—timing of origin, spatial spread, and genetic/distance ratio—is not conclusive when considered separately, taken together, they support the hypothesis that a "consistently moving wave of ZEBOV infection" recently spread to outbreak sites in Gabon and Congo. Following its current course, ZEBOV may hit populated areas east of Odzala National Park within 1–2 years and reach most parks containing large populations of

western gorillas in 3–6 years. Two Ebola outbreaks have already hit human populations west of Odzala, and over the past two years, the largest gorilla and chimp populations in the world, found in Odzala, have been devastated—the disease is spreading to the last unaffected sector of the park right now. These findings suggest that strategies to protect villagers and some of the last remaining wild apes from future outbreaks would do best to concentrate efforts at the front of the advancing wave—and start acting now.

—Liza Gross

Walsh PD, Biek R, Real LA (2005) Wave-like spread of Ebola Zaire. DOI: 10.1371/journal.pbio.0030371

Antisocial Behavior in Cooperative Bacteria (or, Why Can't Bacteria Just Get Along?)

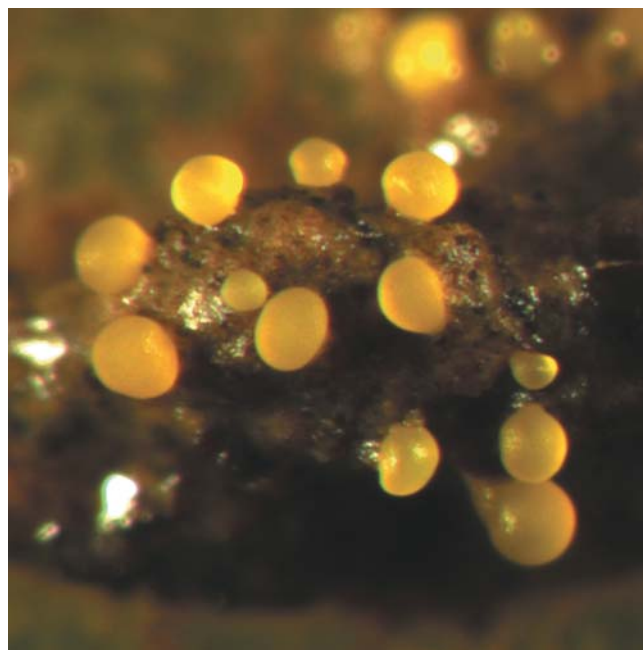
DOI: 10.1371/journal.pbio.0030398

Bacteria are defined as unicellular organisms, but they don't typically function as single cells in nature. Social behavior among bacteria is well established, and makes a lot of sense when you consider that billions of bacteria—representing as many as 1,000,000 species—can be found in just one gram of fertile soil. Cooperative bacteria coordinate a range of complex behaviors through a density-dependent mechanism called quorum sensing: when bacterial numbers reach a critical mass, individual cells secrete signaling molecules that control the behavior of the colony. Through quorum sensing, individual cells amass into biofilms (bacterial colonies that exude slime and other molecules that help them stick to everything from ship hulls to teeth), and some species are able to form structures called fruiting bodies to weather nutrient-poor conditions.

One group of bacterial species, known as the myxobacteria, exhibit several sophisticated social behaviors. They socially swarm and hunt other microbes in a manner analogous to wolf pack hunting. Even more dramatically, when cells of the species *Myxococcus xanthus* fall upon hard times due to lack of food, some 100,000 individuals band together and form fruiting structures. This process is marked by distinct gene expression programs, differentiation, and morphological changes. Inside the fruiting body, rod-shaped cells differentiate into spherical, stress-resistant spores designed to wait out a famine. But only a portion of the population turns into spores; the vast majority either commit cell suicide, making the ultimate sacrifice, or remain undifferentiated.

But how far does this cooperative behavior go? One species of bacteria can comprise many divergent strains, with different genotypes. It's been shown that when two distinct *Myxococcus* species are mixed together, the species segregate and form separate fruiting bodies, with one species dominating the other in spore production. Would mixing divergent strains of the same species produce similar results? In a new study, Francesca Fiegna and Gregory Velicer investigated this question using nine strains of the "highly social" ubiquitous soil bacterium *M. xanthus* isolated from different regions of the world.

To see how divergent strains behave in mixed company, Fiegna and Velicer placed the divergent strains in nutrient-poor cultures, pitting every possible combination of one strain



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Some 100,000 *Myxococcus xanthus* cells amassed into a fruiting body with spores, above. Experimental competitions showed that some strains of this social bacterium exploited others. (Image: Michiel Vos)

against another. After starving the mixed cultures for five days, the authors observed each pair's fruiting body formation, as well as the spore production of each strain in the mixtures and in isolation.

The shape, size, and distribution of fruiting bodies were different for nearly every mixed pair relative to their clonal cultures, with most pairs producing fewer fruiting bodies than each strain in isolation. Mixing also decreased the overall social productivity (indicated by total spore production) of the pairs, with some antagonistic pairs reducing total spore production as much as 90%. Even though most strains responded poorly

to mixing, some performed better in competition than in isolation—revealing that naturally occurring social bacteria are capable of exploiting their neighbors.

Fiegna and Velicer went on to rank the dominant strains (that is, determined which strain produced the most spores), based on the possible pairing interactions, and showed that their fitness ratings were largely hierarchical, with only one case of a rock-paper-scissors (circular) fitness relationship among any three strains out of 82 such comparisons. This hierarchy suggests that diversity would be quickly lost if all nine strains resided together in one mixed population, with only one strain (or a small number of strains) dominating and eliminating the others over time. Thus, these strains do not tend to act as cooperative subunits when mixed, and *M. xanthus* as a species has diverged into multiple, distinct social types that cooperate with clone-mates (and perhaps close relatives) but have no qualms about exploiting distant relatives of the same species.

Since *M. xanthus* can travel great distances carried by water, wind, and an array of animals and insects, the authors conclude, it's possible that resulting antagonisms between introduced foreign strains and resident bacterial populations might decimate some native populations. The degree to which this type of mixing occurs in nature is an active area of research. With the help of whole-genome sequencing and molecular techniques, scientists can refine their traditional morphological classifications of this social soil bacterium to better understand its distribution and likely encounters in soil communities—whether the fitness hierarchies seen here are more typical of mixed distant rather than local strains, for example—and to begin unraveling the molecular agents of subjugation. —*Liza Gross*

Fiegna F, Velicer GJ (2005) Exploitative and hierarchical antagonism in a cooperative bacterium. DOI: 10.1371/journal.pbio.0030370

A Well-Studied Disease-Resistance Gene Shows No Signs of Selection

DOI: 10.1371/journal.pbio.0030400

When our ancestors switched from hunting and gathering to farming about 10,000 years ago, they unwittingly unleashed new selective pressures associated with different diets, increased population density, and novel infectious diseases. This period of rapid change, it is widely thought, also precipitated many genetic adaptations, some conferring resistance to disease. One gene presumed to have undergone positive selection has generated significant interest because of its role in HIV infection. The gene encodes a chemokine receptor called CCR5 on the surface of white blood cells that, along with CD4, mediates HIV entry into the cells.

In 1996, independent research groups discovered a 32-base pair deletion, called $\Delta 32$, in the gene's coding region that confers HIV resistance to individuals with two copies of the gene variant, or allele, and delayed AIDS progression to those with one copy. The mutation was relatively common among northern Europeans but virtually absent in non-Caucasians. In a 1998 study, researchers estimated the mutation's age roughly 700 years by analyzing the genetic variation patterns of 192 Caucasian chromosomes. Because it is unlikely for a young mutation to reach a high population frequency by chance alone, it was thought that some selective agent—alternately thought to be bubonic plague and smallpox—had accelerated its spread.

But now Pardis Sabeti, Eric Lander, and their colleagues report that the mutation may be much older than previously thought—and find no evidence of positive selection. The 1998 study based CCR5 $\Delta 32$'s age, in part, on evidence that the allele was inherited along with two genomic markers, called microsatellites, positioned farther away than would be expected under neutral evolution. Higher than expected linkage disequilibrium (LD)—the distance between linked sequences—suggests the linked sequences are under positive selection. LD shrinks over time because the recombination that occurs during sperm and egg cell development reshuffles sequences around the mutation. Using recently available genome-wide sequence variation data, Sabeti et al. show that the LD and pattern of genetic variation at the allele's locus isn't unusual when compared to the rest of the genome.

For their study, Sabeti et al. analyzed the CCR5 $\Delta 32$ polymorphism, two microsatellites, and 70 single nucleotide polymorphisms (SNPs) on Chromosome 3, where the mutation resides, in 340 chromosomes from European, Chinese, and African (Yoruba, Nigeria) populations. They compared the genetic

diversity at CCR5 to genomic regions from two large empirical datasets: 2,359 SNPs in 168 immunological genes, located across the genome studied in the same 340 chromosomes, and 63,149 SNPs on Chromosome 3 studied for the same populations from the International Haplotype Map Consortium project. The frequency with which CCR5 variants appeared within and between populations was not unusual compared to the 168 genes or to other regions of Chromosome 3. For the gene to be under selection, it should have shown either decreased or increased diversity within a population or greater variation in distribution among populations. As for CCR5 $\Delta 32$'s higher frequency in European populations, that isn't unusual either, it turns out: many other polymorphisms found in similar frequencies (7%–9%) in Europeans do not occur in the other populations.

To estimate the LD around CCR5 $\Delta 32$, Sabeti et al. first applied the technique used in the 1998 study, and similarly found that chromosomes with the mutation have much longer LD than chromosomes without the mutation. But when the authors analyzed the full range of variation at the CCR5 locus, rather than simply examining $\Delta 32$ versus non- $\Delta 32$ variation, they found two variants with longer LD than $\Delta 32$. They further compared the LD around CCR5 $\Delta 32$ to LD around other similarly prevalent mutations on Chromosome 3, and found that it was not unusual. Finally, Sabeti et al. used the new sequence variation data to remap the microsatellite markers, and showed that they're much closer to the mutation than previously thought, pushing the mutation's age back to roughly 5,000 years ago.

These results show that genetic variation around CCR5 $\Delta 32$ is not so different from the rest of the genome, and find no sign of recent selection for the allele. The absence of evidence is not evidence of absence, but the study raises the important issue that evidence for selection should now be examined and re-examined in a genome-wide context. As more genome-wide datasets become available, scientists will be able to compare the pattern of variation at every gene with overall genomic variation. And by finding the signs of selection in our genes, these tools can point to the evolutionary events that shaped our history and shed light on the genetic roots of disease resistance. —*Liza Gross*

Sabeti PC, Walsh E, Schaffner S, Vavilys P, Fry B, et al. (2005) The case for selection at CCR5 $\Delta 32$. DOI: 10.1371/journal.pbio.0030378



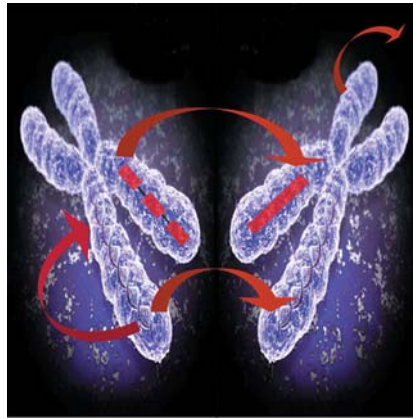
Retrocopied Genes May Enhance Male Fitness

DOI: 10.1371/journal.pbio.0030399

“Retrocopied” genes were long viewed as evolutionary dead ends, with little functional relevance. Such a gene copy is generated by a circuitous route. First, a normal gene is copied to make a messenger RNA, which in the usual scheme of things is sent out of the cell nucleus, used to code for protein, and eventually destroyed. Once in a great while, though, the messenger RNA is “reverse transcribed,” coded back into a DNA sequence by the enzyme reverse transcriptase. It can then be inserted back into a chromosome, quite possibly a different chromosome than the one on which its parent gene resides. This process, which is driven by the legions of transposable elements that litter the genome, usually creates a functionless “retropseudogene,” stranded without a promoter and unable to be expressed.

But occasionally, the new gene copy recruits a promoter by, as yet, largely unknown mechanisms, and may thus potentially become functional, able to code for a protein. In the primate lineage, four such genes have been identified to date. In this issue, Henrik Kaessman and colleagues announce the discovery of seven more, and estimate that on average, one such new gene arises every million years. Many of these genes are expressed predominantly in the testes, where at least some of them probably substitute for X-chromosome genes that are inactivated during sperm development.

The authors first used bioinformatics methods to identify almost 4,000 retrocopies in the human genome. Retrocopied genes can be distinguished from their parents because the introns, or noncoding sequences, of the parent are edited out of the original messenger before retrocopying; thus, the DNA coding sequence is initially the same, but lacks the intervening introns of the parent. Of these 4,000, about 700 had not been disabled by mutations that interrupt the coding sequence. The number of other harmless mutations each had accumulated was then used to estimate the time each retrocopy was formed, based on molecular evolution theory that such neutral mutations occur at a predictable rate. While retrocopies have been created continuously over many millions of years of mammalian evolution, the authors’



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Approximately one new retrogene (a re-inserted copy of a gene) per million years emerged on the primate lineage leading to humans. (Image: © 2005 Hybrid Medical Animation)

analysis showed a peak around 40 million years ago, after the emergence of primates, but before establishment of the human line. They estimate that 57 functional retrogenes arose in primates, about one per million years of primate evolution.

To pinpoint individual retrogenes, the authors first conducted an evolutionary simulation to estimate, based on sequence changes, which retrocopies were likely to still be functional. They found seven, which originated between 18 and 63 million years ago. These genes

play a variety of roles in transcription and translation, as well as chromosome condensation and segregation, which occur just prior to cell division.

They next looked at expression patterns for these genes in 20 human tissues, and discovered that for all seven, expression was restricted mostly or entirely to the testes. Three of the seven genes were copied from genes on the X chromosome to Chromosome 1, 5, and 12, respectively. The authors suggest that such retrogenes functionally replace their silenced parental genes on the X chromosome during spermatogenesis, a resourceful maneuver that may enhance the reproductive fitness of the organism expressing them. This increase in fitness, in turn, preserves the functional retrocopy through natural selection. For two other genes, the authors also infer a function in spermatogenesis based on the parental gene function. One of these genes as well as the two remaining genes appear to have been selectively driven to evolve new or more adapted functional properties compared to their parents. Together, the results suggest that retrogenes were often recruited during primate evolution to enhance male germline functions. —Richard Robinson

Marques AC, Dupanloup I, Vinckenbosch N, Reymond A, Kaessmann H (2005) Emergence of young human genes after a burst of retroposition in primates. DOI: 10.1371/journal.pbio.0030357

New Environments Set the Stage for Changing Tastes in Mates

DOI: 10.1371/journal.pbio.0030389

In the evolutionary war of the sexes, females choose their mates while males fight for the right to inseminate. Darwin explained this widely observed phenomenon in terms of energy expenditure: whichever sex invests more to produce and rear offspring gets to choose. That lot typically falls to females, whose mating preferences have driven the evolution of secondary sex characteristics as diverse as the peacock’s extravagant tail and the fiddler crab’s outsized claw. Such preferences may also influence speciation by causing reproductive isolation, acting as a behavioral barrier to gene flow between populations in much the same way mountain ranges act as physical barriers. In both cases,

isolated populations that once interbred can diverge into separate species.

The effect of sexual selection on speciation has been demonstrated in many different organisms, but it’s not so clear which evolutionary mechanisms—genetic drift or natural selection—account for the initial shift in mating preferences that generate divergent sexual selection. Different mating preferences could arise as a by-product of chance events related to unique mutations (genetic drift) that produce arbitrary traits later modified by sexual selection, or as a side effect of changes in traits that arise as populations adapt differently to their local environments (divergent natural selection). If divergent selection affects



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An evolutionary experiment that manipulated the resource environment of Australian fruitflies (shown mating on a strawberry) showed that female mating preference can evolve, at least in part, in correlation with the environment. (Image: A. O'Toole, University of Queensland)

female mating preferences (assuming that mating preference differences contribute to reproductive isolation), then separate populations that adapt to different environments should also diverge in mating preferences, while populations adapted to similar environments should not.

Working with the Australian fruit fly *Drosophila serrata*, Howard Rundle, Mark Blows, and their colleagues at the University of Queensland in Australia investigated the role of divergent selection in the evolution of female mating preferences. Mate choice in *D. serrata* is mediated by nonvolatile pheromones in the insect's outer cuticle, called cuticular hydrocarbons (CHCs). In past experiments, male CHCs had been shown to evolve rapidly in response to changes in selection (which is not surprising since they protect the fly

against environmental vagaries), but the consequence for female mating preferences was not known.

To address the effect of divergent selection on the evolution of female mating preferences, the authors created different environments in the lab by raising four duplicate fly populations on three different food resources—with yeast representing the ancestral lab environment (these flies have eaten yeast since the stock was established in 1998), and rice and corn representing two novel environments. Flies were raised for 22 months, then fed yeast for two generations to control for environmental effects, before both CHCs and female mating preferences were estimated for each of the 12 populations.

To estimate female mating preferences, a single female from one of the experimental populations was placed in a vial with two males from the ancestral stock population, providing standard males for comparison of preferences among the populations. An average of 106 trials were conducted for each of the populations. After females had mated with one of the two males, CHCs from the chosen and rejected males were extracted for analysis. Female mating preferences were then determined for each population by calculating sexual selection gradients that related the mating success of the males with their CHCs.

CHC profiles for all the flies revealed that nearly every CHC molecule had adapted to the novel environments, although CHC evolution was greater in females than in males. Surprisingly, however, the mating trials showed that

female mating preferences had also diverged consistently among populations in correlation with their environment (preferences were similar among populations from the same environment, but differed among populations from different environments). This so-called parallel evolution, the authors argue, implicates divergent selection over drift in preference evolution because genetic drift is unlikely to produce a pattern of preference evolution that is predictable by environment.

Altogether, the authors conclude, this evolutionary experiment shows that mating preferences “can evolve at least in part in correlation with the environment.” This result is consistent with the classic by-product model of speciation, in which new species arise as a side effect of divergent selection; in this case, mating preferences act as a premating isolation mechanism that arises along with the divergent environments. Interestingly, the authors found no correlation between the CHCs that adapted most and those for which female preferences changed. Teasing apart the relative contributions of natural and sexual selection in the evolution of CHCs and mating preferences may help shed light on the complicated relationship between trait and preference evolution in general—and on the role that preference plays in the emergence of new species.

—Liza Gross

Rundle HD, Chenoweth SF, Doughty P, Blows MW (2005) Divergent selection and the evolution of signal traits and mating preferences. DOI: 10.1371/journal.pbio.0030368

Social Opportunity Produces Brain Changes in Fish

DOI: 10.1371/journal.pbio.0030390

For many animals, possibly even for humans, mating success is determined by social status or dominance. A male's position in the “pecking order”—a term coined by the Norwegian Thorleif Schjelderup-Ebbe, who first proposed the concept of social dominance based on his work with chickens—can actually control his fertility. Social status also has other well-established, long-term physiological consequences. It can determine how big an animal grows, for example, or how it responds to stress. However, little is known about the neural mechanisms that link the social environment to the physiological changes associated with dominance.

Sabrina Burmeister, Erich Jarvis, and Russell Fernald are investigating these neural mechanisms in the cichlid fish *Astatotilapia (Haplochromis) burtoni*, in which dominance is tightly coupled to reproductive physiology. Subordinate males are less sexually mature, lack the bright blue or yellow body coloration and eye stripe that advertises dominance, and do not exhibit dominance behaviors such as territorial defense and courtship. Interestingly, the reproductive capacity of these male cichlids is socially regulated throughout life. A subordinate male can climb the cichlid social ladder

if the dominant male in his social group dies as a result of predation or disease or if a subordinate stages a cichlid coup and dethrones the dominant male. The subordinate's sexual capacity then increases under the control of neurons in the preoptic area of the hypothalamus (a brain region that links the nervous system to hormonal systems), which enlarge and increase their expression of a peptide needed for reproduction called gonadotropin-releasing hormone 1 (GnRH1).

In their new research, Burmeister et al. have concentrated on the behavioral and genomic responses of



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Dominant male *Astatotilapia burtoni* dispute a territorial boundary. (Image: Russell D. Fernald and Sabrina S. Burmeister)

subordinate male cichlids presented with an opportunity to ascend in status. The authors simulated natural social upheavals by removing the dominant male an hour before daylight (cichlids rely primarily on visual cues to monitor their social position) from a social group consisting of several females, a dominant male, and a subordinate male—an approach designed to represent naturally occurring behavioral and neural responses. The authors then watched the erstwhile subordinate male

for behavioral signs of dominance, and measured changes in the expression of a class of genes called immediate-early genes within the brain. The proteins encoded by these genes—which show behavior-specific expression in many animals—induce the expression of other genes in the brain that produce changes in the animal’s physiology. Subordinate and dominant males whose position in the social hierarchy had not been experimentally manipulated were similarly examined.

The researchers discovered that subordinate males become dominant within minutes of an opportunity to do so, rapidly developing the bright coloration of dominant males and indulging in dominant behaviors. Over the same timescale, the expression of the immediate-early gene *egr-1* increased in the anterior preoptic region, peaking in those regions with the highest densities of *GnRH1*-expressing neurons. None of these changes occurred in unmanipulated, or stable, subordinate males. Furthermore, *egr-1* expression did not increase in stably dominant males, even though these fish showed similar behaviors to the ascending males, including the acquisition of dominance coloration, which is not maintained in the dark. In other words, the social opportunity itself rather than any behavior exhibited in response to the opportunity induced *egr-1* expression.

The *egr-1* gene encodes a transcription factor that is important for neural plasticity, and the *GnRH1* gene contains a binding site for *egr-1*. Thus, Burmeister et al. concluded, the social opportunity–induced expression of *egr-1* in the anterior preoptic area is an early trigger in a molecular cascade that ultimately produces the physiological changes associated with dominance in cichlids. Given that the preoptic region and *GnRH* are highly conserved in vertebrates, a similar neural mechanism could link social status to sexual physiology in other animals. —Jane Bradbury

Burmeister SS, Jarvis ED, Fernald RD (2005) Rapid behavioral and genomic responses to social opportunity. DOI: 10.1371/journal.pbio.0030363

Temperature Regulates the Zebrafish Clock

DOI: 10.1371/journal.pbio.0030379

The conveniences of modernity are not without their costs, as weary travelers know all too well. When you jet into a country halfway around the world and it’s dark hours before your body expects nighttime, your internal clock doesn’t have time to adjust, so you feel jet-lagged. The biological clock, calibrated to daily light and temperature cycles, controls the circadian rhythms of a wide range of physiological and behavioral processes, from fluctuating hormone levels to sleep–wake cycles and feeding patterns. While it’s well known that circadian clock elements sense and respond to light cycles, evidence of temperature-dependent

changes in multiple cellular processes—such as gene transcription, translation, and protein stability—in fruit flies and fungi suggest that many circadian clock components also respond to temperature cycles.

Daily temperature cycles and spikes can reset the clock’s phase (timing of the peaks and troughs of activity), though its cycle length remains fixed over a wide range of temperatures. This “temperature compensation” feature confers a measure of resistance to the potentially disruptive effects of temperature fluctuations on the accuracy of the clock’s timing mechanism, and appears to be a general property of the circadian system.

Since little is known about how vertebrates manage these temperature-related responses at the genetic and molecular level, Kajori Lahiri, Nicholas Foulkes, and their colleagues decided to study this question in zebrafish. This genetically tractable model organism is especially suited to this task, the authors explain, because adults, larvae, and even embryos can tolerate a wide range of core body temperatures (being cold-blooded animals) that can be manipulated simply by changing the water temperature. Temperature variations of as little as 2 °C (35.6 °F) can reset the zebrafish clock, Lahiri et al. show, and precise shifts in temperature trigger

significant changes in the expression of specific clock genes.

To test whether temperature cycles can establish, or entrain, circadian rhythms in zebrafish like light–dark cycles do, Lahiri et al. raised zebrafish larvae in total darkness for six days, starting four hours after fertilization, and exposed them to a 4 °C temperature cycle. A subset of fish (at the larval stage) were sacrificed every three hours to measure RNA levels of core clock genes (*per2*, *per4*, *cry2a*, *cry3*, and *clock1*) and determine their expression profiles. As a control, sibling larvae were exposed to light–dark and constant temperature. Clock genes *per4*, *cry2a*, *cry3*, and *clock1* showed rhythmic expression under both light–dark and temperature cycles, with the high temperature phase matching the light phase. Remarkably, the authors wrote, the results were similar for larvae raised with a daily temperature fluctuation cycle of as little as 2 °C.

Zebrafish cell lines also proved valuable tools for studying temperature response, showing a similar pattern of clock gene expression under a 4 °C temperature–darkness cycle as the larvae did under a 2 °C temperature–



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Several key transcriptional regulatory elements at the core of the zebrafish circadian clock respond to temperature.

darkness cycle. Expression of *per4* continued even after the cells were exposed to constant temperature, an indicator of entrainment. Temperature shifts can also trigger significant changes in clock gene expression (transcript levels of *per4* and *cry3* dropped after a temperature increase and spiked after a temperature decrease; *cry2* showed the opposite response)—changes wrought by temperature-dependent shifts in the behavior of

transcriptional regulators, as in the case of *per4*. Acute shifts in temperature alter the expression of several clock genes selectively. How these gene-expression responses fit into this temperature-triggered pathway is unclear, but Lahiri et al. offer a few hypotheses for future testing—investigations that should benefit from using the zebrafish cell lines the authors developed.

The authors go on to show that the zebrafish clock functions over a range of temperatures with characteristic temperature compensation. They speculate that this may result when temperature changes produce shifts in the amplitude of circadian transcription rhythms. Altogether these results show that temperature can regulate the circadian clock in this vertebrate. If the temperature-induced transcriptional responses described here operate in other temperature-related responses, they may shed light on how temperature affects other biological systems as well.

—Liza Gross

Lahiri K, Vallone D, Gondi SB, Santoriello C, Dickmeis T, et al (2005) Temperature regulates transcription in the zebrafish circadian clock. DOI: 10.1371/journal.pbio.0030351

Genetic and Behavioral Investigations into Odor Discrimination and Memory

DOI: 10.1371/journal.pbio.0030388

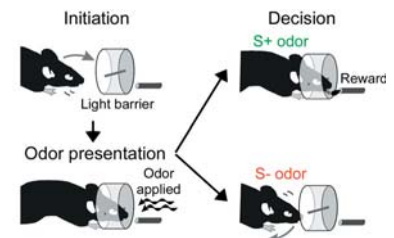
The smell of baking bread, the perfume of flowers, the tang of sea air—your nose can sense and distinguish between these smells and thousands more. The sense of smell—managed by the olfactory system—is a crucial tool for sensing the environment. Thousands of low molecular weight molecules bind to a vast repertoire of odor receptors on olfactory sensory neurons in the nose. These neurons extend long projections into an area of the forebrain known as the olfactory bulb, where chemical messengers (neurotransmitters) pass on information to other neurons elsewhere in the brain. In ways that are only just beginning to be understood, all this information is integrated by neural circuits in the brain so that different odors can be learned and discriminated; in addition, changes in neuron activity are responsible for remembering odors.

The neural circuits that underlie odor learning and discrimination and olfactory memory rely on neurotransmission that is mediated by ion channels (pores that allow ions to pass through the

normally impermeable cell membrane) called γ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPA). Each AMPAR is comprised of multiple subunits of glutamate receptors (GluRs), which form the ion channels. Most AMPARs contain GluR-B, which controls many of their properties, including their permeability to calcium ions.

Derya Shimshek, Andreas Schaefer, and their colleagues are combining genetic studies and quantitative behavioral studies to assess how AMPARs contribute to olfactory learning, discrimination, and memory. To investigate these processes, the researchers have constructed two sets of genetically modified mice. In the first set, some neurons in the forebrain express a form of GluR-B that increases the calcium ion permeability of AMPARs. In the second set, GluR-B expression is partially reduced in the forebrain, a manipulation that also renders AMPARs calcium permeable.

In behavioral tests, in which mice were rewarded for their ability to discriminate between similar test odors, genetically



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Water-deprived mice are trained to distinguish a water-rewarded odor (S+) and an unrewarded odor (S-) by their licking response.

altered mice showed quicker olfactory learning and increased discriminatory prowess than mice without genetic alterations in GluR-B. Thus, increased AMPAR-mediated entry of calcium ions into neurons within the forebrain, in particular within the olfactory bulb, seems to enhance olfactory learning and discrimination. By contrast, GluR-B-depleted mice showed impaired odor memory. Different GluR-B-depleted mice had different degrees of memory

impairment (even though they all had similar improvements in odor learning and discrimination). This variability, the researchers suggest, could reflect regional differences in the expression of residual GluR-B produced by the genetic manipulations used to derive the mice. When the scientists investigated this idea by measuring GluR-B expression in various areas of the brains of mice whose behavior they had already tested, they found that the decreases in olfactory memory in individual GluR-B-depleted mice strongly correlated with reductions in GluR-B levels in their hippocampus and piriform cortex. Taken

together with data from other groups that discounts the involvement of the hippocampus in the type of long-term olfactory memory tested here, this result strongly supports the prevalent view that the piriform cortex is important in olfactory memory.

Overall, the results presented by Shimsek and colleagues suggest that olfactory discrimination and memory in mice are achieved in mechanistically and spatially distinct ways even though the same ion channel receptor is involved. The researchers' experimental approach—combining quantitative behavioral analyses with genetic

manipulations that introduce variable patterns of gene expression into the brain—should prove invaluable for dissecting the neural circuitry underlying not only olfaction but also other sensory systems in mice. And because these systems are very highly conserved, finding out about mouse olfaction should also indicate how humans sniff out good and bad smells. —*Jane Bradbury*

Shimsek DR, Bus T, Kim J, Mihaljevic A, Mack V, et al. (2005) Enhanced odor discrimination and impaired olfactory memory by spatially controlled switch of AMPA receptors. DOI: 10.1371/journal.pbio.0030354

Arp2/3: Structural Insights into a Primary Engine of Cell Motility

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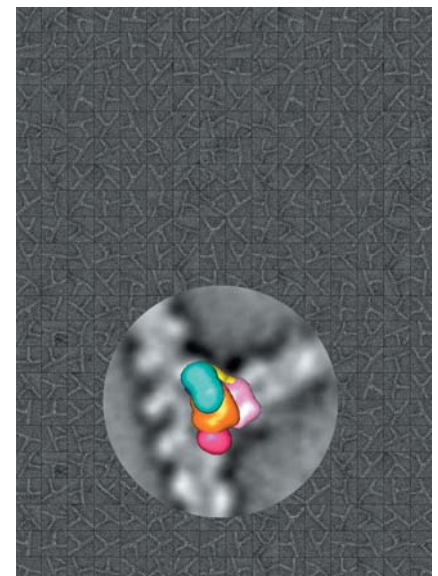
The capacity for directed motion arises from core cellular processes shared by organisms from protozoa to primates, indicating an ancient origin over a billion years ago. Amoeba foraging for food, pathogenic microbes invading host tissue, host immune cells ingesting those pathogens—all depend on the same core motility components. Actin, the most abundant protein in eukaryotic cells (cells with nuclei), lies at the heart of the motility machinery. Individual actin proteins assemble into polymers and organize into long filaments that form intricate networks under the direction of a seven-subunit protein complex called the actin-related protein (Arp) 2/3 complex. As actin polymerization occurs, newly formed daughter filaments grow out at an angle from mother filaments, much like branches sprout from a tree trunk. The place where daughter filaments bud from a mother filament (branching nucleation) is called the branch junction.

Branch formation requires Arp2/3 activation, which occurs when the Arp2/3 complex binds to nucleation promoting factors (which includes a family of proteins called WASp), molecules of adenosine triphosphate (ATP)—a molecule that fuels most energy-requiring processes—and already-existing mother filaments. The details of branch junction formation have remained obscure, though nucleation models have proposed that Arp2 and Arp3 assemble around the mother filament and form a template for actin subunits to nucleate from. While the atomic structure of the inactive Arp2/3 complex (previously determined) shows that the two actin-

related proteins resemble actin enough to form a template for nucleation, the spatial relationship between the proteins in the inactive complex does not match that in actin filaments.

Current models postulate that Arp2/3 complex activation triggers a change in spatial relationship so that Arp2 and Arp3 resemble actin monomers in a filament; but without evidence on the structure of the Arp2/3 subunits at the branch junctions, these models have to assume that this is how nucleation occurs. In a new study, Coumaran Egile et al. combine electron microscopy, genetic labeling, and computational analysis to resolve the structure of the Arp2/3 complex at the branch junction to a resolution of one nanometer (that's one billionth of a meter), and demonstrate that the Arp2/3 template assumption is correct.

To study the mechanics of branched actin nucleation by the Arp2/3 complex, Egile et al. assembled actin branches in test tubes and observed the action at the molecular level. To do this, the researchers tagged the different Arp2/3 subunits with labels that can be detected with electron microscopy, allowing them to determine the location of the proteins. Using this approach, they introduced different nucleation promotion factors—WASp proteins as well as cortactin (an Arp2/3 activator that is found near the inner cell membrane)—and compared the resulting branch junction formations. Only cortactin was found at the branch junction, supporting the model that WASp activators transiently bind, activate, and release Arp2/3 after branch formation. Cortactin, on the other hand, may stay behind to help stabilize the



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The actin-related protein (Arp) 2/3 complex provides a template for new actin filaments to branch off from a mother filament.

interaction between Arp2/3 and the mother filament.

After genetically engineering yeast to express fluorescently labeled versions of the different subunits, Egile et al. observed the complexes' nucleation activities and located their position in the branch junction. The likely orientation of the subunits at the branch junction was determined with computational modeling. Given the position of the subunits and the number of possible combinations at this site, the authors used the crystal structure of the inactive Arp2/3 complex to map all the possible orientations. Only one cluster of orientations satisfied the constraints: Arp2 and Arp3, associated with the fast-

growing end in an actin filament, facing away from the mother filament and toward the daughter filament.

Though Arp2 and Arp3 orientations would have to change upon activation to support daughter filament growth, the authors argue that the change would not disrupt the overall architecture of the complex. Rotating the subunits to reflect their activated conformations places Arp3 next to the mother filament and Arp2 farthest away. In this orientation,

the longest axis of the complex aligns perpendicular to the mother filament (in all previous models, they align parallel), an arrangement that could provide stability at the branch junction, by taking advantage of protein interactions on either side of the mother filament.

Altogether, these results provide conclusive evidence for the starting assumption of a nucleation model in which Arp2 and Arp3 form a template at the branch junction that triggers

daughter filament growth. And with the help of the subunit map presented here, researchers can further dissect the molecular mechanisms of actin branch nucleation and elucidate the dynamics of cellular motion. —*Liza Gross*

Egile C, Rouiller I, Xu XP, Volkman N, Li R, et al. (2005) Mechanism of filament nucleation and branch stability revealed by the structure of the Arp2/3 complex at actin branch junctions. DOI: 10.1371/journal.pbio.0030383

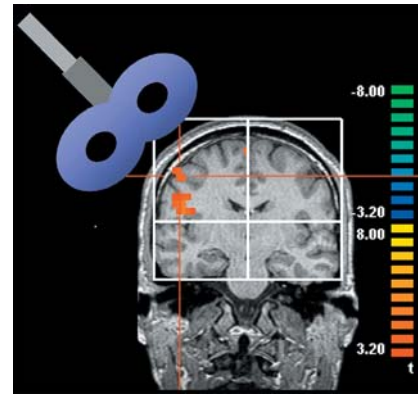
Stimulating the Brain Makes the Fingers More Sensitive

DOI: 10.1371/journal.pbio.0030408

Repetitive transcranial magnetic stimulation (rTMS) has more than a whiff of Buck Rogers to it: a magnetic wand passes over the surface of the skull, triggering changes in the brain underneath. But it's not science fiction, and in the past decade, rTMS has emerged as an intriguing technique for exploring brain function, and a promising, though still unproven, form of therapy. In this issue, Hubert Dinse, Martin Tegenthoff, and their colleagues show that a short course of rTMS can increase finger sensitivity for up to two hours after treatment ends, and that this change corresponds to an increase in the size of the brain map representing the finger.

rTMS is applied with an electromagnetic coil in the shape of a figure-eight, placed on the scalp directly over the targeted portion of the brain. Short bursts of a strong magnetic pulse stimulate electrical currents within. Sensory input from each region of the body is represented on the surface of the brain, and the location of any region—in this case, the right index finger—can be mapped to allow precise targeting of the rTMS. The authors adjusted the strength of the magnetic field to just below that which triggered a sensory response in the finger, and then applied intermittent pulses of stimulation over the course of about ten minutes.

They tested the sensitivity of the index finger by determining how far apart two simultaneously applied pinpricks needed to be for the subject to distinguish them



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Transcranial magnetic stimulation alters sensory perception and activity in sensory cortical areas.

as separate stimuli. rTMS increased this two-point discrimination by about 15% immediately after stimulation, an effect that gradually diminished but still remained significant over the course of the next two hours. The effect was fairly specific for the right index finger: there was no effect on the left index finger, which is represented in the opposite hemisphere, and only a small effect on the right ring finger, which is represented several millimeters away from the index finger in the same hemisphere. When stimulation was applied over the area representing the lower leg, the index finger did not become more sensitive.

The authors used functional magnetic resonance imaging (fMRI) to see how the brain changed in response to the

stimulation. They found that the region representing the index finger got larger, and that the degree of increase in any one subject corresponded to the degree of increased sensitivity in that same subject. As the sensory effect faded, so too did the fMRI changes. Thus, the cortex itself undergoes changes as a result of rTMS.

Practice affects the brain and the brain affects practice—it now appears possible to directly intervene in this brain-behavior loop to improve short-term tactile performance. Other recent work by the same authors shows that rTMS can also improve visual discrimination, suggesting a potential for affecting changes throughout the brain. These results are unlikely to be of immediate benefit to those who rely on exquisite sensitivity in their fingers, whether surgeons or safecrackers, as the equipment needed for rTMS is cumbersome and the duration of the effect relatively short. However, a related technique, transcranial direct current stimulation, employs much more portable equipment, suggesting it may find a role in neurorehabilitation. Further study of both techniques will be needed to determine the future of this futuristic technology. —*Richard Robinson*

Tegenthoff M, Ragert P, Pleger B, Schwenkreis P, Förster AF, et al. (2005) Improvement of tactile discrimination performance and enlargement of cortical somatosensory maps after 5 Hz rTMS. DOI: 10.1371/journal.pbio.0030362