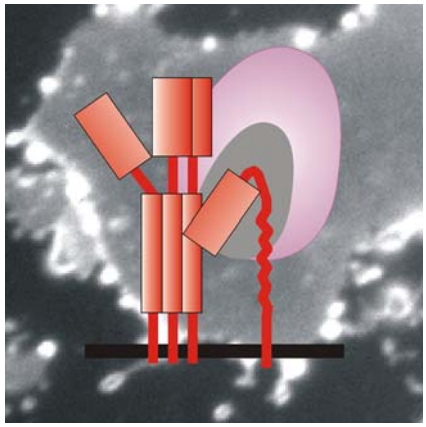
A Flexible Syntaxin Solves the Mystery of the SNAREd Munc

Richard Robinson | DOI: 10.1371/journal.pbio.0040359

Fusion of two plasma membranes is central to exocytosis, the process by which a cell secretes neurotransmitters, digestive enzymes, and other products. If you believe the simple diagrams in introductory biology textbooks, you'd think this fusion occurs as soon as two membranes touch. Not so—in fact, membrane fusion requires interaction among a complex set of proteins in the membranes, collectively termed SNARE proteins. SNAREs are assisted by a second group, called SM proteins, which bind to them and help promote their functions.

Among the SM proteins, one, called Munc18-1, has stood out as something of an oddball. When the others bind to their respective SNAREs, they leave the SNAREs in an open conformation, available for interacting with others and forming the complexes that drive membrane fusion. In contrast, Munc18-1 appears to fold its SNARE, syntaxin 1, into a closed conformation, making it unavailable for binding to other SNAREs. But this result has been obtained only in membrane-free solutions, and the behavior of membrane-bound Munc18-1 has been a mystery. A new study by Felipe Zilly, Thorsten Lang, and colleagues resolves the mystery of the syntaxin–Munc18-1 interaction and explains how their binding promotes interactions with other SNAREs.

The authors performed their experiments in sheets of membrane, prepared by disrupting cells, which mimic the native biochemical environment of Munc18-1 much better than membrane-free solutions. First they showed that syntaxin must be able to close to bind Munc18-1; when mutated to prevent closure, syntaxin bound virtually no Munc18-1. But proteins are flexible molecules, and it is possible that syntaxin needn't stay closed while it is bound to Munc18-1, and that adopting a more open conformation while bound would promote its linkage to other SNAREs.



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Munc18-1 (purple) binds to a half-open syntaxin (red), which is primed to form SNARE complexes with synaptobrevin and SNAP-25 in plasma membrane sheets (seen in the background image).

To test this possibility, the authors added another SNARE, synaptobrevin, to the mix. Synaptobrevin is a known partner for syntaxin, and it has been shown that the addition of synaptobrevin drives syntaxin (without Munc18-1) in conjunction with SNAP-25 (the third SNARE essential for neuroexocytosis) into SNARE complexes. They reasoned that adding synaptobrevin to syntaxin–Munc18-1 would not drive syntaxin into SNARE complexes if syntaxin remained closed. Conversely, if syntaxin could partially open while bound to Munc18-1, it would be able to interact with synaptobrevin and join the SNARE complex. And this is what they found—when synaptobrevin was added, syntaxin unhitched from Munc18-1 and joined the SNARE complex, involving most likely also SNAP-25. Finally, by deleting SNAP-25, the authors verified its essential role in displacing Munc18-1 from syntaxin, suggesting there is an intermediate complex formed by Munc18-1, syntaxin, synaptobrevin, and SNAP-25.

These results not only shed light on the actual function of Munc18-1, but allow the development of a more coherent picture of SM proteins, in which Munc18-1 is no longer the oddball. They also illustrate the complex interactions among proteins that bring about such a "simple" process as membrane fusion.

Zilly FE, Soerensen JB, Jahn R, Lang T (2006) Munc18-bound syntaxin readily forms SNARE complexes with synaptobrevin in native plasma membranes. DOI: 10.1371/journal.pbio.0040330

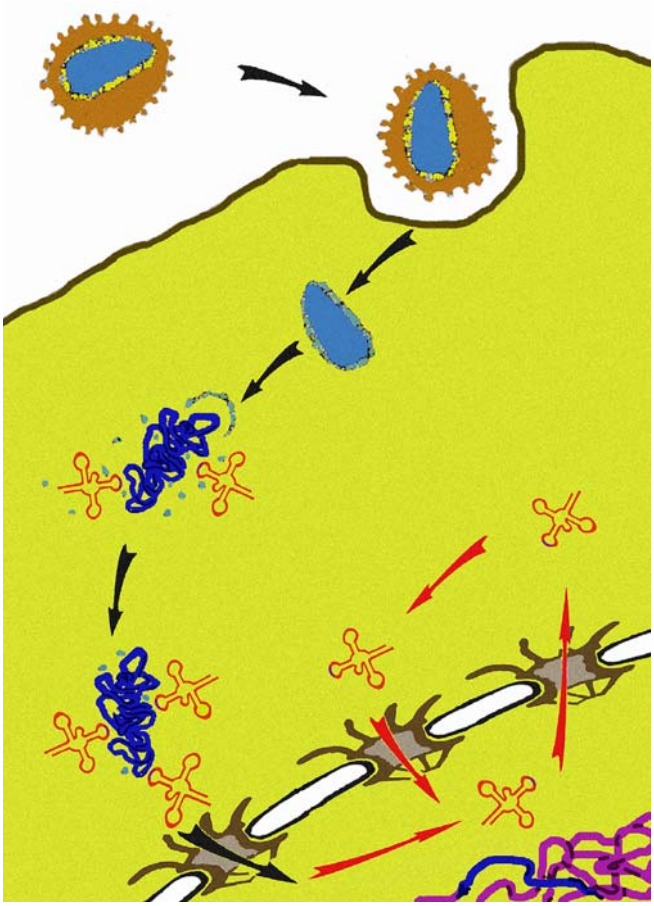
Nuclear Import of HIV-1 Complex Relies on a Surprise Player: tRNA

Richard Robinson | DOI: 10.1371/journal.pbio.0040364

To complete its life cycle, it is not enough for HIV-1, the virus responsible for AIDS, to get inside a cell. It must also integrate into the host cell's DNA. In a dividing cell, the virus can get access to the cell's DNA when the nuclear envelope dissolves during mitosis. But in cells that do not divide, such as the macrophages of the immune system, the virus must somehow insert its "reverse transcription complex" (RTC) into the nucleus. The RTC contains the virus's RNA genetic material, as well as proteins that will copy it into DNA and integrate the copy into the host genome.

That it does get into the nucleus has been known for some time, but the details of how it does so are still mysterious. In a new study, Lyubov Zaitseva, Richard Myers, and Ariberto Fassati reveal a surprising key player—the host cell's own transfer RNA (tRNA). During protein synthesis, tRNA matches amino acids to messenger RNA.

Attempts have been made to study these details before by systematically mutating the virus and identifying genes and sequences critical for successful nuclear import. But the results have not been clear-cut, since on the one hand, mutations often alter more than one character of the virus, and on the other, there may be multiple pathways for nuclear import, not all of which are disrupted by single mutations.



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HIV-1 exploits retrograde transport of tRNAs in human cells to promote nuclear import of its reverse transcription complex. (Image: Lyubov Zaitseva)

To circumvent these problems, the authors took a different approach. They isolated RTCs and labeled them with a fluorescent tag that allows the authors to track the complexes. They then introduced the RTCs into cells whose cytoplasm had been removed, leaving only the cell's cytoskeleton and nucleus in place. Using a high-speed centrifuge, they separated cytoplasm from cultured cells into fractions and tested each fraction for its ability to promote nuclear import of the RTCs in the gutted cells. As expected, when they added in the fraction containing several known nuclear transport factors, the RTCs could enter the nucleus. But even after these known factors were removed from the fraction, it still promoted nuclear import, indicating the presence of other, unidentified players.

By further purifying and characterizing the active fraction, the authors showed that the molecules responsible were small RNAs, whose sequences were very close to standard human tRNAs. The small RNAs differed from the standard variety in several ways, possibly reflecting defects in their synthesis. In particular, they lacked a terminal triplet of nucleotides, CCA, which normally binds an amino acid, and whose absence appeared to be critical for the import process; full-length normal tRNAs promoted import only poorly, and when the authors added CCA ends to synthesized versions of their newly identified tRNAs, the import ability of the tRNAs fell. The authors also found that these damaged tRNAs were incorporated into budding virus particles—which may use tRNAs for the next round of infection—and that this activity was promoted by one of the virus's proteins. Finally, and most remarkably, they found that nuclear import of damaged tRNAs did not require RTC but did require cellular energy, suggesting it is a normal cellular process, not just a consequence of HIV-1 infection.

The discovery of tRNA-mediated import of RTCs helps to further characterize the life cycle of this important and grimly fascinating virus. But perhaps equally interesting is the discovery that nuclear import of tRNA occurs at all and that it appears to be a standard part of the cell's repertoire. A similar transport activity has recently been seen in yeast, and some evidence suggests it may be a mechanism for sequestration and repair of damaged tRNAs; by removing damaged tRNAs from the cytoplasm, protein synthesis may continue without interference. But it is also possible that undamaged tRNAs enter (and quickly re-exit) the nucleus; further study will be needed to explore these possibilities.

Zaitseva L, Myers R, Fassati A (2006) tRNAs promote nuclear import of HIV-1 intracellular reverse transcription complexes. DOI: 10.1371/journal.pbio.0040332

Chance Fluctuations in mRNA Output in Mammalian Cells

Françoise Chanut | DOI: 10.1371/journal.pbio.0040319

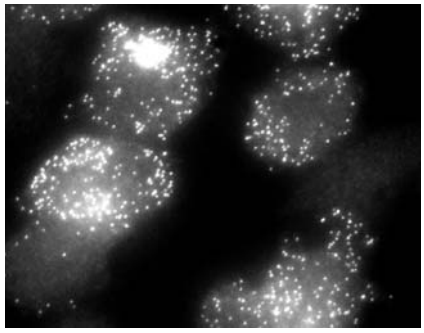
In the drama of cell biology, both genetics and environment write the script, and chance throws in the twists of plot. In general, most cells live relatively predictable lives: divide, differentiate, and die. Yet chance leaves its imprint even in ordinary cells. For instance, bacterial or yeast cells in culture are known to produce widely different amounts of certain proteins, even when they are genetically identical. Scientists attribute such cell-to-cell variations to chance fluctuations in the cells' ability to make these proteins. They also speculate that such fluctuations may benefit the cells in their struggle to adapt and survive.

But opinions vary as to which step in protein production is subject to random fluctuations. Proteins result from the translation of messenger RNAs (mRNAs), which come from the transcription of genes. Fluctuations in protein output could reflect a whimsy intrinsic to the expression of the coding gene, or random swings in global, or extrinsic, regulators of the gene's expression. In yeast, extrinsic causes, such as variations in cellular volume, seem to predominate. But in a new study, Arjun Raj and his colleagues show that in mammalian cells, intrinsic causes, specifically the genes' ability to transition randomly between active and inactive transcriptional states, can be crucial.

The researchers developed a sensitive technology to detect mRNAs in single cells and examined the output of a genetically engineered reporter gene they called M1, which they had introduced into Chinese hamster ovary (CHO) cells. After staining cultures of identical cells with a fluorescent probe specific to M1, they counted

fluorescent dots corresponding to single M1 mRNA molecules in individual cells. At any given time, a few cells displayed a large, bright cluster of these spots, indicative of recently transcribed mRNAs still densely packed around the M1 gene. These cells also had the largest number of M1 mRNAs (over 150). In these cells, the M1 gene was therefore actively churning out mRNAs. But the majority of cells displayed fewer than 50 M1 mRNAs that were dispersed over their entire volume. In these cells, the M1 gene had become silent. These results showed that the M1 gene is expressed via infrequent bursts of transcriptional activity in the CHO cells.

If these bursts reflected the uneven distribution of transcription factors among CHO cells, then multiple reporter genes in a single cell should burst at the same time. The researchers generated cell lines that contained two versions of the reporter gene (M1 and M2) that were controlled by the same transcriptional activators but whose mRNAs could be distinguished with probes of different colors. In some lines, the two genes had landed in separate genomic locations. The researchers found that they burst independently of each other. They concluded that bursts are not induced by extrinsic factors such as M1- or M2-specific transcriptional activators. Rather, they reflect the intrinsically random ability of both the M1 and M2 genes to switch between an inactive and an active state.



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Individual reporter mRNA molecules are shown in a field of clonal CHO cells. Though genetically identical, the cells display extreme variations in their expression levels.

Nevertheless, when the two versions of the reporter gene landed next to each other, they did burst in synchrony, as if switching spread locally among contiguous genes. This observation suggests that switching follows the waves of condensation and decondensation that randomly breathe through the coils of genomic DNA (chromatin). Indeed, genes are mostly silent when packed into dense chromatin. But when they decondense, the transcription machinery can latch on to their DNA and begin making RNA. Raj and his colleagues propose that genes switch to an active state as a consequence of randomly initiated decondensation, and that transcription factors merely stabilize their active state. Consistent with this proposal, they find that reducing the availability of a transcriptional factor, or increasing M1's affinity for this factor, does not significantly increase the frequency of bursts. Only the size of the bursts—that is, the amount of RNA produced in each burst—increases.

Random bursting is not restricted to artificial genes such as M1. The researchers document the same behavior in the gene encoding RNA polymerase, the lead actor of transcription. That cells would tolerate chance fluctuations in an mRNA so fundamental to their survival comes as a surprise. The researchers show that in the case of the M1 gene, protein stability buffers the consequences of erratic mRNA production. This is because stable proteins are only "topped up" by the occasional bursts of transcription, whereas unstable proteins follow the variations in mRNA levels more closely. This finding indicates that protein stability may be a critical factor in the cell's ability to tolerate variations in transcription. Whether chance fluctuations in RNA production sometimes produce beneficial twists of fate for mammalian cells remains to be shown.

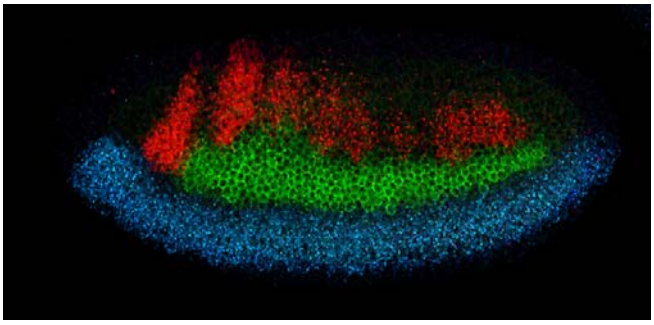
Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S (2006) Stochastic mRNA synthesis in mammalian cells. DOI: 10.1371/journal.pbio.0040309

BMPs: Conserved Morphogens in Neural Patterning

Françoise Chanut | DOI: 10.1371/journal.pbio.0040346

During evolution, organisms seem to maintain the function of certain key genes but vary the mechanisms that call these genes into action. For instance, fruit flies and vertebrates share three genes—*vnd*, *ind*, and *msh*—that induce distinct cell fates in the developing nervous system. Both flies and vertebrates express these genes in three adjacent stripes that span the length of their nascent nerve cords. But stripe patterns in fruit flies depend on the nuclear protein Dorsal and in vertebrates on the signaling molecule Hedgehog; both factors are produced in ventral regions of the nerve cord. Since Dorsal and Hedgehog belong to unrelated signaling pathways, vertebrates and fruit flies seem to have independently evolved distinct means of expressing *vnd*, *ind*, and *msh* in the same striped pattern. But in a recent study, Claudia Mieko Mizutani, Ethan Bier, and colleagues demonstrate that both flies and vertebrates similarly rely on a third dorsally produced signal, the bone morphogenetic proteins (BMPs), to paint stripes along their nerve cords. They propose that BMPs are the ancestral patterning signal that the precursors of flies and vertebrates originally shared and that later were reinforced by recruiting either Dorsal or Hedgehog in neural development.

BMPs are extracellular proteins that function early in embryonic development to stem neural differentiation and promote epidermal cell fates. As a result, the nervous system of flies and vertebrates arises only where other extracellular proteins known as Chordin or Sog stave off the influence of BMPs. But in many developmental processes, BMPs act as morphogens: they diffuse away from their source and create patterns in neighboring tissues by regulating gene expression in a dose-dependent manner. As development proceeds, Chordin and Sog subside while the BMPs continue bathing the epidermis. Mizutani thought that the BMPs might also act as morphogens in the developing nerve cord, but that their role would be masked by the more prominent effect of Dorsal or Hedgehog. Thus, to unmask any possible effect, the researchers erased all trace of Dorsal or Hedgehog pattern.



DOI: 10.1371/journal.pbio.0040346.g001

Localized inhibition of BMP signaling in an early *Drosophila* embryo leads to a dorsal shift in the expression domains of neural identity genes. (Image: Claudia Mieko Mizutani)

In flies, the Dorsal protein has a graded distribution that peaks at the embryo's ventral midline and tapers off midway around the embryo's circumference. High Dorsal concentrations elicit expression of *vnd* in two ventro-lateral stripes (one on each side of the embryo), while intermediate Dorsal doses turn on *ind* at a middle location. The dorsal *msh* stripes lie next to the source of Dpp, the fly BMP, which emanates from the embryo's dorsal half. By manipulating the genetic control of Dorsal distribution, Mizutani and her colleagues created embryos with a uniform, intermediate level of Dorsal protein, and no Dpp. In these lateralized embryos, all patterning by Dorsal is erased: *ind* is expressed around the whole embryo's circumference, and *vnd* and *msh* remain silent.

When the researchers genetically introduced a local source of Dpp in lateralized fly embryos, part of the stripe pattern was recreated: *ind* was repressed locally and made room for *msh* expression close to the Dpp source. This relative pattern of *msh* and *ind* expression is similar to that in normal embryos and indicates that Dpp represses expression of *ind* more effectively than *msh*. While this experiment shows that Dpp can act as a morphogen in the fly nervous system, it doesn't demonstrate that Dpp is actually required to do the same thing during normal development. To address this question, the researchers created local disruptions of Dpp signaling in normal embryos by expressing Brinker—a nuclear protein that blocks Dpp signaling—in a narrow stripe perpendicular to the *vnd*, *ind*, and *msh* stripes: all three stripes swerved dorsal-ward when they crossed Brinker. This result confirms that Dpp normally patterns the fly's nervous system by limiting the dorsal expansion of the *vnd*, *ind*, and *msh* stripes.

To test whether BMPs function analogously in patterning of the nervous system in vertebrates, co-authors Henk Roelink and Néva Meyer ventralized fragments of chick neural tubes by soaking them in a Hedgehog solution. The fragments originally expressed only *Nkx2.2*, *vnd*'s vertebrate homolog. But when cultured next to a tissue fragment releasing BMPs, the fragments activated the intermediate and dorsal neural genes *Pax6* and *Msx* in concentric rings, recapitulating the expression pattern seen in normal neural tubes. Hence, in vertebrates as well, BMPs alone can recreate most of the dorso-ventral pattern of neural gene expression. This suggests a conserved role for BMP function during early and late patterning of the neuroectoderm. This unified view in which BMPs repress neural genes in a dose-dependent fashion runs contrary to the prevailing view of vertebrate neural patterning, since BMPs have been proposed to turn on genes in dorsal regions of the neural tube.

A morphogen shared by flies and vertebrates is likely to come from their common ancestor, which was small, according to fossil data. The researchers speculate that a BMP gradient may have been sufficient to pattern the nerve cord of a small organism. But as organisms grew larger, the need arose for additional patterning mechanisms to complement the BMPs, which eventually lost their preeminence in nervous system patterning.

Mizutani CM, Meyer N, Roelink H, Bier E (2006) Threshold-dependent BMP-mediated repression: A model for a conserved mechanism that patterns the neuroectoderm. DOI:10.1371/journal.pbio.0040313

Transposon Silencing Keeps Jumping Genes in Their Place

Liza Gross | DOI: 10.1371/journal.pbio.0040353

Nearly a century ago, two geneticists described "rogue" pea plants with an unorthodox pattern of inheritance. William Bateson and Caroline Pellew found that crossing inferior rogues with normal plants always produced rogue offspring, suggesting that the rogue appearance was a dominant trait. The real surprise came when rogue progeny were crossed back to normal plants. Following the principles of Mendelian inheritance, these crosses should have produced a mix of normal and rogue plants, but they produced only rogue plants. The phenomenon, later dubbed "paramutation," allowed the rogues to break the rules by acting "epigenetically"—inducing heritable changes in gene expression without DNA mutations. In one-sided interactions between gene pairs, or alleles, only "paramutagenic" alleles can attenuate, and eventually silence, the expression of "paramutable" alleles.

Epigenetic silencing involves chemical modifications to DNA and the histone proteins that remodel the chromatin surrounding DNA, rendering genes inaccessible to transcription-related proteins. Epigenetic silencing also targets "transposons," genetic elements that can jump around the genome. Both paramutagenic alleles and transposons contain tandem or inverted repetitive DNA sequences. Recent work in a variety of species suggests that such repeats can induce heritable silencing when they trigger the production of double-stranded RNAs, which are then processed into small interfering RNAs that can inactivate genes through DNA methylation and other mechanisms.

A new study by Margaret Roth Woodhouse, Michael Freeling, and Damon Lisch sheds light on these somewhat mystifying processes by identifying a gene that keeps both transposons and paramutated color genes silenced in maize, confirming results recently published in *Nature*. The gene, *Mediator of paramutation1 (Mop1)*, encodes an RNA-processing enzyme called RNA-dependent RNA polymerase 2 (RDR2) that is required to make the small RNAs needed to maintain silencing of a transposon

(called *MuDR*). While *Mop1* maintains *MuDR* silencing, the authors show that a second gene is required to establish heritability of silencing.

MuDR can be heritably silenced by a paramutagenic gene called *Mu killer (Muk)*—an inverted repeat variant of the *MuDR* transposon. *MuDR* encodes two genes—*mudrA* causes excision of the element and, with *mudrA*, *mudrB* helps reinsert it. *Muk* produces hairpin double-stranded RNAs that trigger rapid processing of full-length *mudrA* into small RNAs, leading to the destruction of *mudrA* transcripts and the methylation and silencing of *MuDR*. *Muk*-induced *MuDR* silencing begins with *mudrA*, then spreads to the adjacent *mudrB* gene.

Mutations in *Mop1* interfere with both *Mutator* transposon methylation and paramutation of several maize color genes. Woodhouse et al. found that *mop1* is an evolutionary cousin of the RDR2 found in *Arabidopsis*, where it effects transposon silencing. Maize plants carrying two mutant copies of *mop1* failed to produce small RNAs corresponding to *mudrA* and *mudrB*—confirming *Mop1*'s role as an RNA-processing enzyme.

To test the *mop1* mutant's effects on *Muk*-induced *MuDR* silencing, the researchers bred plants that carried both *Muk* and *MuDR* in the presence or absence of a functional copy of the *Mop1* gene. Mutant plants showed clear evidence of *MuDR* silencing, suggesting that *Mop1* is not required to initiate silencing. These plants also continued to produce small RNAs specifically associated with the initiation of silencing. Thus, although *Mop1* is required to make the small RNAs associated with the maintenance of *mudrA* and *mudrB* silencing, it is not required to make the small RNAs associated with the initiation of *Muk*-induced *MuDR* silencing. Further, the progeny of these mutant plants carried only inactive *MuDR* elements, indicating that *Mop1* is not required for heritable silencing of *MuDR*—though there appear to be other factors that are required for this process. Offspring of plants that had both *MuDR* and *Muk* but lacked functional nucleosome assembly protein 1 (NAP1, a chromatin-building protein) gave rise to heavily spotted kernels—the sign that heritable *MuDR* silencing had been disrupted. Yet the loss of NAP1 did not block the *initiation* of silencing, because these plants had methylated *Mutator* elements whether or not they expressed NAP1, which suggests that *mudrA* activity had been lost in both cases. Thus, while losing NAP1 didn't prevent *Muk* from initiating *MuDR* silencing, it did prevent *Muk* from establishing a stable, heritably silenced state.

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Heavy spotting on corn kernels reveals the activity of the *Mutator* system. (Photo: Damon Lisch)

Altogether, these results show that distinct factors initiate, establish, and maintain *MuDR* silencing. *Muk* initiates silencing by targeting *mudrA* with its hairpin RNAs, leading to the destruction of mudrA transcripts and methylation of the transposon's terminal inverted repeats. NAP1 is required to establish heritable silencing, likely by changing chromatin into a transcription-unfriendly state. Mop1/RDR2 then maintains silencing by using RNA processing to mediate continued DNA methylation.

Given the damage that transposons can cause by inserting themselves into essential genes, it's not surprising that organisms have evolved enduring mechanisms to keep jumping genes in their place. This study contributes a valuable framework for identifying the factors that regulate the enigmatic epigenetic processes that defend the genome against invasive elements—and helps explain how these changes can persist and be transmitted to the next generation.

Woodhouse MR, Freeling M, Lisch F (2006) Initiation, establishment, and maintenance of heritable MuDR transposon silencing in maize are mediated by distinct factors. DOI: 10.1371/journal.pbio.0040339

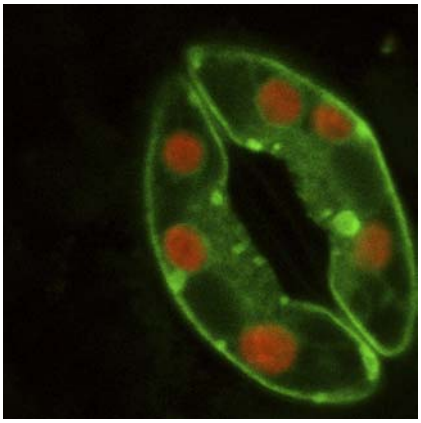
Protein Kinases and Plant Pores

Liza Gross | DOI: 10.1371/journal.pbio.0040358

When water is scarce, plants synthesize a hormone that facilitates conservation by closing stomatal pores on their leaves. Each pore is surrounded by a pair of guard cells that control stomatal aperture in response to various stimuli, including the drought-triggered hormone called abscisic acid (ABA). ABA signaling increases calcium levels in guard cells; calcium in turn acts on a variety of channels that regulate the transport of ions across the cell membranes. As both positively and negatively charged ions (called anions) cross the membrane, turgor pressure drops and stomata close.

These observations support a model in which ABA signaling includes calcium signaling. But ABA signaling also affects parallel pathways and mechanisms—including raising pH levels—and no mutations in calcium-sensing proteins have been previously reported that positively transduce an ABA response in plants. Thus, identifying the molecules that sense and transduce calcium signals in guard cells would provide valuable insights into the mechanics of ABA signaling. Calcium-dependent protein kinases (CDPKs) are calcium-sensor candidates, but with 34 CDPK genes in *Arabidopsis* alone, functional redundancy in this enzyme family has likely thwarted efforts to characterize their contribution—that is, when the function of one is disrupted, another can step in to fill its role.

In a new study, Izumi Mori, Julian Schroeder, and colleagues describe two CDPK genes—*cpk6* and *cpk3*—with clear roles in calcium and ABA signaling in guard cells. (In previous guard-cell microarray experiments, Schroeder



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Confocal image of an *Arabidopsis* stomate showing two guard cells exhibiting green fluorescent protein and native chloroplast (red) fluorescence. (Image: Alex Costa)

and colleagues had narrowed down the number of guard cell–expressed CDPK genes to a more manageable number.) Losing function of the cpk3 and cpk6 genes in guard cells impairs ABA- and calcium-induces activation of a class of anion channels (slow, or S-type) and stomatal closure. Elevated calcium levels activate S-type anion channels through phosphorylation—a chemical reaction that regulates protein activity; kinases typically function by phosphorylating target proteins.

Plant biologists can investigate gene function by inserting DNA (called transferred DNA, or T-DNA) from the soil bacterium *Agrobacterium tumefaciens* into a plant's genome. When the inserted T-DNA disrupts a gene's function, researchers can infer gene function based on observed defects in plants carrying the mutant genes, or alleles. After confirming that the *cpk3* and *cpk6* alleles were in fact function-disrupting mutants in *Arabidopsis* plants, Mori et al. sequenced the alleles and identified two different insertion mutations for both alleles *(cpk3-1* and *cpk3-2*, and *cpk6-1* and *cpk6-2)*. Then they isolated single mutant plants, with two copies of just one allele, and double mutants, with two copies of different combinations of the alleles (for example, two copies of both *cpk3-1* and *cpk6-1* or of *cpk3-2* and *cpk6-2*), for further study.

All the mutant plants looked normal, though both double mutants grew a bit behind schedule compared to the nonmutant (wild-type) plants. ABA-induced stomatal closure, however, was partially impaired. The researchers examined the mutants' effect on calcium and ABA activation of anion channels. In wild-type guard cells, elevated calcium levels activated large S-type anion channel currents, but this activation was significantly reduced in both *cpk3* single mutant cells and even more so in single *cpk6* mutants. Reduced currents were also observed in both double mutants, though they did maintain a background anion current. Double mutants also exhibited reduced ABA activation of the S-type anion channels. Interestingly, Mori et al. also found that ABA activation of another class of ion channels, calcium-permeable channels, was impaired in the single and double *cpk* mutants, revealing the first genetic mutants that impair both ABA regulation of calcium channels and calcium activation of anion channels. Thus, CDPKs play an important role in calcium-mediated ABA regulation of S-type anion channels, calcium channels, and stomatal closing.

This study provides direct genetic evidence that calcium sensors function in stomatal ABA signaling and that CPK3 and CPK6 function as ion channel regulators in guard cell signaling. Because stomatal closing was partially preserved in cells lacking these kinases and another class of "(rapid) R-type" anion channels was less affected in the *cpk* mutants, the authors further conclude that parallel calcium-dependent and -independent signaling mechanisms are at play in a branched guard-cell signaling network. Using a cell-specific signaling and protein regulation approach, as described here, researchers can begin the tall task of characterizing responses of gene-disruption mutants in other members of the large CDPK family function throughout the plant kingdom.

Mori IC, Murata Y, Yang Y, Munemasa S, Wang Y, et al. (2006) Calcium-dependent protein kinases CPK6 and CPK3 function in abscisic acid regulation of guard cell S-type anion- and Ca^{2+}-permeable channels and stomatal closure. DOI: 10.1371/journal.pbio.0040327

Infection Status Drives Interspecies Mating Choices in Fruit Fly Females

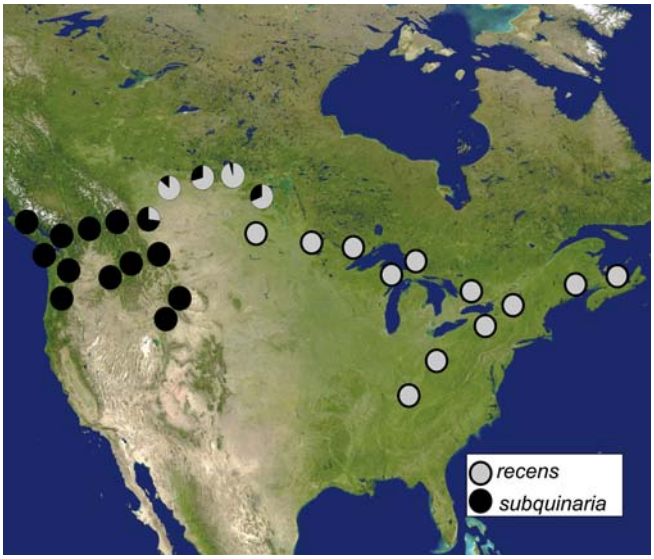
Richard Robinson | DOI: 10.1371/journal.pbio.0040345

Hybridization is a constant possibility for two closely related species. Geographic isolation prevents interbreeding in some cases, but when the range of the two overlap, other mechanisms must come into play if they are to remain genetically distinct. Behavioral isolation is one such mechanism. If members of each group preferentially mate with their own kind, the two species can remain distinct even while residing together. Over time, such isolating behaviors may become more pronounced, and the genes governing them more widespread, a phenomenon termed "reinforcement."

In evolutionary theory, reinforcement has typically been thought to act symmetrically on the two species. In a new study, however, John Jaenike and colleagues show that bacterial infection of one *Drosophila* species, but not another, and the resulting differences in hybrid viability, may account for highly asymmetrical reinforcement occurring in the two.

Wolbachia is a bacterium that infects many insect species, where it lives within the cells of the host, especially the ova and testes, and is transmitted from infected females to their offspring. *Wolbachia* infects virtually all members of the fruit fly species *Drosophila recens*, but not members of the closely related *D. subquinaria*. When an infected male *D. recens* mates with an uninfected female *D. subquinaria*, most offspring die in a process called cytoplasmic incompatibility. In contrast, however, when an infected female *D. recens* mates with an uninfected male *D. subquinaria*, the offspring are viable, and the hybrid females are fertile (the males are sterile, a typical result from cross-species hybridization).

To explore the effect of this difference on reinforcement, the authors began by establishing that the two species do indeed overlap in part of their range (a condition called sympatry), in central Canada, while maintaining separate populations elsewhere (allopatry). In the laboratory, uninfected *D. subquinaria* females from the region of sympatry



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Geographical distributions of *Drosophila subquinaria* (black) and *D. recens* (gray), showing allopatric populations of *D. subquinaria* in the west and *D. recens* in the east, and sympatric populations in central Canada.

never mated with *D. recens* males, while those from the region of allopatry did. They found no such pattern for infected *D. recens* females; instead, females from both regions were likely to mate with uninfected *D. subquinaria* males when placed together. The same discrimination or its lack was seen whether the females were presented with only one type of male (no choice conditions), or with males from both species, as might occur in the wild.

These mate-choice experiments illuminated two important phenomena. First, the most discriminating *D. subquinaria* females were those from populations living side-by-side with infected *D. recens* males. This makes sense, the authors suggest, given that less-choosy females that engage in such matings would leave few offspring, since almost all die off. Indeed, as the authors discovered, sympatric *D. subquinaria* females appeared to be so averse to mating outside their group that they also avoided mating with *D. subquinaria* males that came from the allopatric region. In contrast, allopatric *D. subquinaria* females, which have not been subjected to the same selective pressure, are not as discriminating. Second, *D. recens* females did not avoid interspecific matings nearly as strongly, since they also are not under the same selective pressure. Thus, the reinforcement process—the increase in mate discrimination—is highly asymmetric between the two species.

Finally, the authors asked whether the behavioral differences between sympatric and allopatric *D. subquinaria* females correlated with larger-scale genetic differences between the groups. They found it did not, and that overall there is considerable gene flow between the populations. This indicates that the differences in mate choice are likely the result of natural selection acting within the region of sympatry, rather than simple genetic isolation of the two populations. Interestingly, the reproductive isolation of the two *D. subquinaria* populations has been driven not by factors intrinsic to them, but by infection of entirely different species. It is possible that this isolation will ultimately lead to speciation within *D. subquinaria*, although the current high degree of similarity and existence of gene flow may suggest otherwise.

Jaenike J, Dyer KA, Cornish C, Minhas MS (2006) Asymmetrical reinforcement and *Wolbachia* infection in *Drosophila*. DOI: 10.1371/journal.pbio.0040325

For Yeast Protein Hubs, More Data Means More Connections

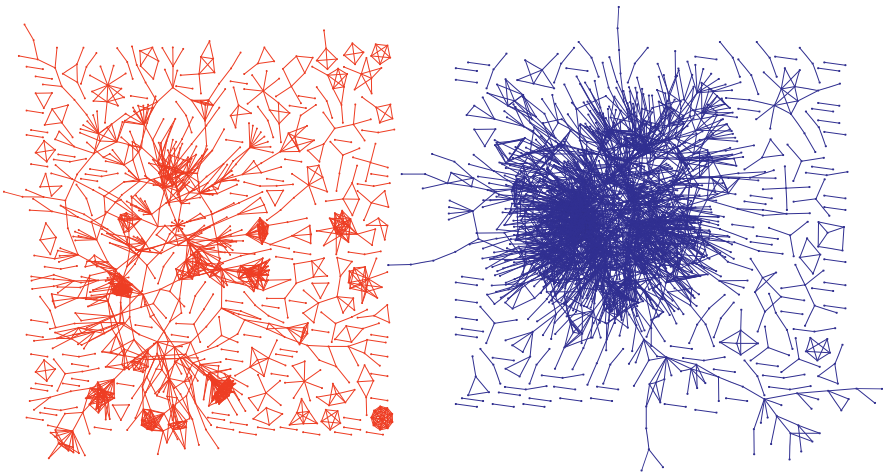
Richard Robinson | DOI: 10.1371/journal.pbio.0040331

What happens inside a cell? To a good first approximation, the answer is "thousands of proteins interact." A cell's form, and all its functions, arises from those interactions. One goal of systems biology is to describe those interactions—by focusing not just on this organelle or that signaling pathway, but on the entire network of proteins within the cell—and then to deduce the patterns of interactions that control that network.

Initial analysis of such protein networks in the budding yeast *Saccharomyces cerevisiae* has led to a hub-centric view of interactions, in which a small number of proteins, the hubs, interact with a disproportionately large number of other proteins. In this model, the hubs form the basis for functional "modules" that perform discrete tasks in the cell. Such modules have been thought to be physically and functionally discrete from other modules, so that there is much interaction within the module, and relatively little between modules. In particular, it has been proposed, hubs of different modules tend not to interact with one another. One version of this model further suggests two types of hubs: "party" hubs are co-expressed and co-localized with most members of their module (together creating a party), while "date" hubs are not, instead engaging in a series of temporally and/or spatially distinct interactions (dates), including interactions with partners in other modules.

Models are only as good as the information they are based on, though. A new study by Nizar Batada, Laurence Hurst, Mike Tyers, and colleagues, combining data from several large budding yeast data sets, shows a much higher degree of interaction between hubs from different clusters, and finds no evidence for the date–party distinction. Thus, the global network appears more homogeneous and perhaps harder to tease into discrete modules than anticipated.

The authors began by integrating data from multiple large data sets of yeast protein interactions, to create a unified set of over 9,000 interactions among almost 3,000 proteins, more than three times as much information on which previous models have been built. As in previous work, certain proteins—the hubs—emerge as being highly connected, binding to and interacting with many more partners than expected by chance. But the previously identified trend for hubs to avoid interaction with one another disappeared in the large data set, a result that persisted as the data were sifted through several different kinds of analytic filters. Hubs



DOI: 10.1371/journal.pbio.0040331.g001

Global organization of yeast interaction networks. The smaller filtered yeast interactome (FYI) network (left) contains locally dense regions that are sparsely interconnected, whereas the larger high-confidence FYI (HC^{fyi}) network (right) is densely interconnected overall, suggestive of extensive coordination and dependencies among diverse processes.

are still real and, according to this result, frequently are among the many proteins they interact with.

Because of the proposed role of date hubs as vital linkages between modules, deletion of date hubs should cause a collapse of the entire network. This result was indeed seen in smaller data sets, and gave support to the initial concept of date hubs. In the larger set, however, no such collapse occurs, as many alternative links still exist between modules even after the deletion of putative date hubs. Neither does the date-versus-party distinction emerge from analysis of co-expression: some hub proteins are co-expressed with their interacting partners more than others, but there is a continuous range from massive co-expression to very little co-expression, not two distinct classes of co-expression behavior. Finally, it has been predicted that date hubs evolve more quickly than party hubs, because their intermodular function allows them more flexibility than a party hub, whose function (it has been argued) is more rigidly fixed by its role within its module. When the authors tested this prediction for yeast protein evolution, no such correlation emerged. Together, these data indicate that the date–party distinction is more likely a property of the small data set it was developed from than a bona fide attribute of the global yeast protein interaction network.

A key finding of this study is that there is a generally higher level of connectivity between clusters that were once thought to be relatively isolated functionally. The authors liken the structure of the earlier model to altocumulus clouds: dense, billowy clouds connected by the thinnest of wisps. A more appropriate analogy, they say, might be stratus clouds: a thick cloud cover with lumps and thinner spots, not uniform but not discrete either. One consequence of this connectivity structure is that functional modules may be harder to physically delineate than has been previously thought, at least in yeast (modules do appear to be more discrete in prokaryotes). Another consequence is that hub–hub interactions, which often reflect essential connections, may form the critical regulatory backbone of the cell.

One alluring feature of the previous altocumulus model of connectivity was that, with few hub–hub interactions, the problem of inadvertent activation of a module by a distant hub was minimized. Instead, the stratus model suggests that such cross-talk may be an important problem for the cell, and that tight control of hub–hub interactions is likely to be a feature of hub regulation; initial evidence suggests this is indeed the case. Further exploration of these and other predictions may clarify the usefulness of the stratus model in developing a systems-level understanding of the cell.

Batada NN, Reguly T, Breitkreutz A, Boucher L, Breitkreutz BJ, et al (2006) Stratus not altocumulus: A new view of the yeast protein interaction network. DOI: 10.1371/journal.pbio.0040317

Gut Bacteria Cospeciating with Insects

Liza Gross | DOI: 10.1371/journal.pbio.0040357

With some 1 million species and counting, insects may be the most abundant class of animals living today. Their protective exoskeleton, prolific reproductive rate, and wings help their cause, as do the symbiotic bacteria that inhabit their cells, gut, or body cavity. Endocellular symbionts live inside specialized insect cells and provide essential nutrients for their hosts, which in turn provide suitable habitat for the bacteria. Insect mothers transmit endocellular symbionts to their offspring during egg or embryo development, preserving an intimate bond between host and symbiont that is evident in both species' genomes.

Studies that use genome analysis to infer evolutionary relationships (called phylogenetics) show that the history of insect host genes (or phylogeny) often mirrors that of their endocellular symbiont—indicating a shared evolutionary history, or cospeciation. Unlike endocellular symbionts, gut or body cavity symbionts are vulnerable to displacement or attack by other microbes and appear to have less-exclusive relationships with their hosts, based on reports that host–symbiont phylogenies for termites and alydid stinkbugs do not match. But a new study suggests that not all gut symbionts go for the promiscuous lifestyle. Takahiro Hosokawa, Takema Fukatsu, and colleagues provide the first evidence of cospeciation between a group of gut symbionts and their insect hosts, plataspid stinkbugs. Not only do their phylogenies mirror each other, but the gut symbionts share many of the unique genetic traits typical of endocellular symbionts.

Plataspid stinkbug symbionts live in the bugs' posterior midgut and are vertically transmitted by the mother in symbiont "capsules." When the female lays eggs, small, brown

symbiont-filled capsules always appear under the egg mass. Nymph hatchlings ingest symbionts from the capsule.

Hosokawa et al. collected 12 populations of stinkbugs, representing three genera and seven species, from several locations in Japan. (Four species were used in the experiments.) All females had the same three-compartment midgut, which had been previously described in two other species: one section contains the symbionts (called the thin crypt-bearing midgut, or TCM), another secretes webbing that embeds the symbionts into the capsules, and a third produces the shell that encases the capsule. All the females also codeposited capsules and egg masses. (Males have only the TCM.)

After removing the TCM from adult females, the researchers analyzed the DNA of the resident bacteria—focusing on a ribosomal RNA gene called 16S rRNA often used to identify bacteria—and found that each bacterial species was associated with a different stinkbug species. Using the 16S rRNA sequences to infer the bacteria's evolutionary origins, they discovered that the sequences didn't match any other bacterial sequences in the databases—they fell into their own class of *Proteobacteria*. Interestingly, however, the symbionts did form a sister group—indicating evolutionary kinship—with the well-characterized obligate endosymbiont *(Buchnera aphidocola)* of aphids.

Given the phylogenetic similarity between the stinkbug symbionts and *Buchnera*, the researchers wondered whether their biology might be similar as well. They divided egg masses into two groups and deprived one group of capsules to generate sibling populations with and without gut symbionts. Adults lacking symbionts showed developmental delays, grew smaller, failed to copulate or reproduce, and died prematurely. Like aphids depend on their endosymbionts, plataspid stinkbugs depend on their gut symbionts to survive—how they do this, however, will be interesting to discover. Like *Buchnera*, the gut endosymbionts also appear to have co-evolved with their host. The phylogenetic tree of the stinkbugs, the researchers found, "perfectly agreed" with the phylogenetic relationships of the gut symbionts. Maternal transmission of the symbiont capsule provides a means of stable transmission, but other factors such as physiological compatibility may come into play.

The symbiotic lifestyle appears to have shaped the genome evolution of endocellular symbionts, which have a small genome, a high percentage of A and T nucleotides in their DNA, and accelerated molecular evolution. Whether these genetic traits arose from population genetic forces—for example, small population size and bottlenecks—or from some aspect of the endocellular environment has been a matter of dispute. Hosokawa et al. found the same "peculiar" genetic patterns in the gut symbionts, lending support to the population genetic hypothesis. They named these gut symbionts "*Candidatus* Ishikawaella capsulata," in honor of Hajime Ishikawa, a pioneer in the molecular study of symbiosis, who recently passed away.

How the symbiont capsule evolved remains an open, and intriguing, question. With some 530 species and 56 genera in the Plataspidae family, researchers have their work cut out for them as they survey the lineages for a stinkbug without a capsule. But with this unique plataspid stinkbug system, they will be well equipped to study insect symbiosis and its influence on genome evolution.

DOI: 10.1371/journal.pbio.0040357.g001

A mating pair of the Japanese common plataspid stinkbug *Megacopta punctatissima*.

Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T (2006) Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. DOI: 10.1371/journal.pbio.0040337

Predicting Species Abundance in the Face of Habitat Loss

Liza Gross | DOI: 10.1371/journal.pbio.0040336

Habitat loss poses the greatest threat to the survival of a species, and often precipitates the demise of top predators and wide-ranging animals, like the Siberian tiger and the orangutan. Any hope of recovering such critically endangered species depends on understanding what drives changes in population size following habitat contraction.

The key question is whether population change is driven directly by changes in habitat volume, or indirectly, through responses to other species of potential predators, prey, and competitors. Ecologists rely on two types of models to predict potential responses to habitat alterations. In single-factor models, population size is controlled by one factor, such as changes in habitat size (as large blocks of forest are fragmented by clear-cutting and development, for example). This is the classic ecological model, in which habitat size drives changes in the abundance of individual species. These models also include "keystone species effects," which look at how populations respond to the loss of a single top predator, like the tiger. In food-web models, species abundance depends on complex interactions across multiple trophic levels, including energy transfer through the food chain.

In a new study, Nicholas Gotelli and Aaron Ellison test the relative contributions of habitat contraction, keystone species effects, and food-web interactions on species abundance, and provide experimental evidence that trophic interactions exert a dominant effect. Until now, direct evidence that trophic

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***Sarracenia purpurea*. (Photo: Nicholas J. Gotelli)**

interactions play such an important role has been lacking, in part because manipulating an intact food web has proven experimentally intractable, and in part because these different modeling frameworks have not been explicitly compared.

Gotelli and Ellison overcame such technical limitations by using the carnivorous pitcher plant *(Sarracenia purpurea)* and its associated food web as a model for studying what regulates abundance in shrinking habitats. Every year, the pitcher plant, found in bogs and swamps throughout southern Canada and the eastern United States, grows six to 12 tubular leaves that collect enough water to support an entire aquatic food web. The pitcher plant food web starts with ants, flies, and other arthropods unlucky enough to fall into its trap. Midges and sarcophagid fly larvae "shred" and chew on the hapless insect. This shredded detritus is further broken down by bacteria, which in turn are consumed by protozoa, rotifers, and mites. Pitcher plant mosquito larvae feed on bacteria, protozoa, and rotifers. Older, larger sarcophagid fly larvae also feed on rotifers as well as on younger, smaller mosquito larvae.

Working with 50 pitcher plants in a bog in Vermont, Gotelli and Ellison subjected the plants to one of five experimental treatments, in which they manipulated habitat size (by changing the volume of water in the leaves), simplified the trophic structure (by removing the top trophic level—larvae of the dipterans fly, midge, and mosquito), did some combination of the two, or none of the above (the control condition). Dipteran larvae and water were measured as each treatment was maintained; both were replaced in the control condition and more water was added in the habitat expansion treatment. These treatments mimic the kinds of changes that occur in nature as habitat area shrinks and top predators disappear from communities.

Gotelli and Ellison counted all the pitcher plant residents through the course of an entire field season in which the treatments were applied to the plants. They next evaluated how well the different models—incorporating different assumptions about habitat, keystone species, and food-web interactions—predicted the observed abundances. Overall, food-web models provided more-accurate indicators of species abundance than simple single-factor models, in which the abundance of each species depends on only one variable. The model based on habitat size alone (that is, the water volume), for example, did not do a good job of predicting individual species' abundances, undercutting the traditional notion that habitat contraction leads to a simple decline in abundance across the board.

The best predictors of abundance were models that incorporated trophic structure—including the mosquito keystone model. This model accurately reflected the pitcher plant food web, with mosquito larvae preying on rotifers, and sarcophagid flies preying on mosquito larvae. "Bottom-up" food-web models (in which links flow from prey to predator) predicted that changes in bacteria population size influence protozoa abundances, which in turn affect mosquito numbers, and that changes in bacteria abundance also affect mite numbers, which impact rotifer abundance. This scenario lends support to the model of a *Sarracenia* food web in which each link in the chain performs a specialized service in breaking down the arthropod prey that is used by the next species in the processing chain.

With over 200 million acres of the world's forestlands destroyed in the 1990s alone, and an estimated 40% increase in the human population by 2050, a growing number of species will be forced to cope with shrinking habitat. Instead of trying to determine how individual species might respond to habitat loss, Gotelli and Ellison argue that incorporating trophic structure into ecological models may yield more-accurate predictions of species abundance—a critical component of species restoration strategies.

Gotelli NJ, Ellison AM (2006) Food-web models predict species abundances in response to habitat change. DOI: 10.1371/journal.pbio.0040324

Disrupted Intercellular Communication Causes a Disfiguring Birth Defect

Liza Gross | DOI: 10.1371/journal.pbio.0040335

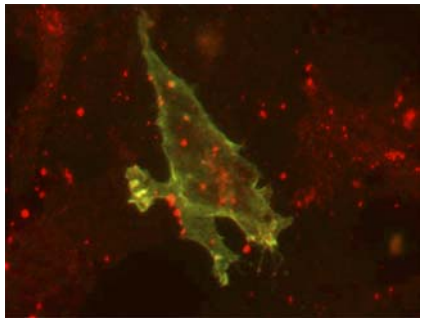
Before a fertilized egg begins the repeated rounds of cell division that turn the single cell into a proliferating, streaming, differentiating mass of cells, its fate may already be sealed. Inherited mutations in genes involved in segregating and sorting embryonic cells can result in serious abnormalities in body patterning and appear to underlie an inherited X-linked disorder (so-called because the mutated genes lie on the X chromosome) called craniofrontonasal syndrome (CFNS). X-linked disorders tend to affect males more severely than females, because boys inherit just one X chromosome while girls inherit two: if one gene is defective, the other can fill in. CFNS is a rare departure from this pattern, with females exhibiting the most severe symptoms. This disfiguring disorder is characterized by a range of skull aberrations, including facial asymmetry, widely spaced eyes, and abnormal head shape, as well as polydactyly and fused digits.

A class of receptor protein-tyrosine kinases called Ephs and their ephrin binding partners (called ligands) regulate tissue patterning by restricting cell interactions, ensuring proper cell sorting, and establishing developmental compartment boundaries. Mutations

in one ephrin gene, *ephrin-B1*, have been identified in patients with CFNS and have been associated with aberrant skeletal patterning in mutant "heterozygous" female mice, which carry one normal and one nonfunctional copy of the *ephrin-B1* gene. Mutations in connexins, structural proteins that form gap junction pores, also lead to cranial and skeletal defects in both mice and humans.

In a new study, Alice Davy, Jeffrey Bush, and Philippe Soriano elucidate the mechanisms of ephrin-mediated cell sorting, and show how the breakdown of the process causes physical abnormalities. The researchers worked with *ephrin-B1* heterozygous female mice, polydactyl mutants with abnormally developed frontal bones in the skull (called the calvarial phenotype, after the name of the bones). They show that Eph/ephrin signaling regulates gap junction communication, which in turn controls cell sorting. Their results indicate that flawed cell sorting, resulting from dysregulated communication at gap junctions—intracellular membrane channels with pores that allow coupled cells to exchange small molecules—underlies the skeletal abnormalities observed in the mice.

Previous studies established that *ephrin-B1* heterozygous females exhibit polydactyly while males lacking their copy of *ephrin-B1* and females lacking both copies do not. Polydactyly accompanied a random inactivation of X chromosomes in female cells (in which one X chromosome is silenced in some cells and the second is silenced in others) that created a mosaic pattern of *ephrin-B1* expression, with *ephrin-B1*-expressing cells segregated from cells that didn't express the gene. Ephrin-B1 mutants also develop multiple defects in tissue derived from neural crest cells—which give rise to cartilage, bone, connective tissue, and other specialized tissues.



DOI: 10.1371/journal.pbio.0040335.g001

Localization of ephrin-B1 (green) and connexin43 (red) in 3T3 cells.

In this study, Davy et al. show that the mosaic loss of *ephrin-B1* blocked the differentiation of neural crest cells by disrupting the distribution of a connexin (Cx43) that regulates bone cell differentiation and forms gap junctional pores. Cx43 aggregated between wild-type (nonmutant) cells and between cells that lack *ephrin-B1*, but was rarely seen at the border between *ephrin-B1*-positive and -negative cells, suggesting that the mosaic cells restricted the number of junctional pores. Expression of the ephrin-B1 receptor, EphB2, is elevated in *ephrin-B1*-negative regions in *ephrin-B1* heterozygous embryos, so the researchers suspected that interactions between the receptor and ligand reduced Cx43 levels and disrupted gap junction formation—which they confirmed by tracking gap junction communication in cell cultures. This defect might be mediated by a physical interaction between ephrin-B1 and Cx43.

The researchers propose that gap junction communication is inhibited by interactions between Eph-positive and ephrin-positive cells that cause Cx43 to be sequestered inside cells, where they can't form gap junction pores and establish cell-to-cell communication—leading to skeletal abnormalities. This explains why the CFNS phenotype is more prevalent in females (who exhibit mosaic expression of *ephrin-B1* through X inactivation). By contributing a mouse model with skull and digit defects that mimic those seen in humans, the researchers have provided a valuable platform for future investigations into the role of ephrins and gap junction communication in disfiguring skeletal disorders.

Davy A, Bush JO, Soriano P (2006) Inhibition of gap junction communication at ectopic Eph/ephrin boundaries underlies craniofrontonasal syndrome. DOI: 10.1371/journal.pbio.0040315

Jack-of-All-Trades "Supergene" Controls Butterfly Wing Pattern Diversity

Liza Gross | DOI: 10.1371/journal.pbio.0040329

While studying the local flora and fauna of the Amazon jungle in the 1860s, Henry Walter Bates made a striking discovery. Butterflies inhabiting a particular geographic region sported the same wing patterns—even though they were unrelated species. Bates proposed that nonpoisonous species had mimicked the patterns of noxious species that predators avoided, thus gaining a selective advantage. (This adaptation is viewed as one-sided; if predators eat the foul-tasting "model" butterfly, they learn to shun all butterflies with that pattern. But if they sample a palatable mimic, they're likely to stop avoiding butterflies with that pattern—until they relearn their lesson.)

Today, scientists can use the tools of genomics and genetics to investigate the mechanism of convergent evolution—the emergence of similar physical traits (or phenotypes) in unrelated species. Such empirical studies have provided insight into a longstanding controversy spawned by evolutionary theorists over the origin of mimicry: does it arise gradually through the accumulation of random mutations by selection or in "phenotypic leaps" through the constraining influence of shared developmental pathways with a bias toward a particular phenotype?

Recent molecular studies found evidence that regulation or recruitment of the same genes or gene variants may explain convergent evolution. In a new study, Mathieu Joron, Chris Jiggins, and colleagues took a different approach by investigating the molecular basis of both convergent and divergent phenotypes. The involvement of the same genomic loci in convergent phenotypes suggests that developmental constraints give rise to these shared phenotypes. The presence of a multitude of convergent and divergent phenotypes in the wing patterns of *Heliconius* butterflies allowed the researchers to test the possibility that mimetic convergence results from constraints in the regulation of butterfly color patterns.

The researchers worked with three species of *Heliconius* butterflies, including Müllerian mimics (all mimetic species

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***Heliconius numata*, f. *silvana* feeding from a *Psiguria* flower. (Photo: Mathieu Joron)**

are poisonous, to mutual benefit). Two species, *H. melpomene* and *H. erato*, are distantly related yet have identical wing patterns. Both species radiated into over 30 races (or subspecies) in parallel, with the two species ("co-mimics) displaying a single pattern locally. The third species, *H. numata*, is closely related to *H. melpomene* but has radically different wing patterns, with up to seven different variations in a single region. Each of these variations mimics a different species of another butterfly genus, *Melinaea*.

In *H. melpomene*, variation in white and yellow patterns is controlled by at least three genomic loci—*N*, *Yb*, and *Sb*—that are tightly linked, or inherited together. Red pattern elements are controlled by another linked loci pair—*B* and *D*. In *H. erato*, the *Cr* locus produces patterning effects similar to the interaction of *N*, *Yb*, and *Sb* in *H. melpomene*, while an unlinked locus, *D*, appears to control red pattern variation much like the *B–D* pair does in *H. melpomene*. In polymorphic *H. numata*, all the mimicked color patterns derive from a single locus, *P*, thought to be a "supergene" (a tightly linked cluster of individual genes).

The researchers crossed different races of each of the three species to explore the genetic basis of the variations. For example, two different subspecies of *H. melpomene* from different regions in Ecuador were crossed with an *H. melpomene* subspecies stock from French Guiana to produce second-generation offspring. Offspring were then genotyped to identify genes responsible for the resulting color patterns and to map the relevant major color-patterning loci—*N*, *Yb*, and *Sb* loci for *H. melpomene* crosses, *Cr* for *H. erato*, and *P* for *H. numata*—in individual offspring.

Using molecular markers developed in the region of the pattern genes, they found that the three loci controlling color pattern variation for each species inhabit the same genomic location. Indeed, the elements controlling white and yellow pattern variation in *H. melpomene* (*N*, *Yb*, and *Sb*) and *H. erato (Cr)* are tightly linked to genetic markers that occupy the same position in both species. Similarly, the locus *P*, which controls whole-wing variation in *H. numata*, is also linked to the same series of markers.

These results, Joron et al. conclude, suggest that a single conserved locus is responsible for producing wing pattern diversity in *Heliconius* butterflies. Rather than a constraining role, this locus provides what the researchers call a "jack-of-all-trades flexibility." It presumably functions as a "developmental switching mechanism" for natural selection, they explain, by responding to a wide range of mimetic pressures to produce radically divergent, locally adapted wing patterns. Now researchers can begin to identify and determine the modus operandi of the genes at the center of what has been called a "developmental hotspot" to better understand how they drive the adaptive evolution of mimetic color pattern shifts. For more on the evolution of mimicry in butterflies, see the Primer (DOI: 10.1371/journal.pbio.0040341).

Joron M, Papa R, Beltrán M, Chamberlain N, Mavárez J, et al. (2006) A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. DOI: 10.1371/journal.pbio.0040303

When Evidence Is Scant, Mathematical Modeling Offers a Roadmap for Discovery

Liza Gross | DOI: 10.1371/journal.pbio.0040323

Scientists often talk about the value of intuition in guiding them toward the big questions. But when it comes to figuring out how all the pieces of a complex system fit together—with hordes of uniquely behaving components interacting in nonlinear pathways—systems biologists prefer to rely on computers. Theoretical analysis has increasingly been applied to understanding protein, gene, and biochemical networks in cells. Most cell network models either generate dynamic, quantitative descriptions (specifying the timing and kinetics of interactions) of well-defined pathways but use relatively few components, or present static maps of protein–DNA or protein–protein interactions that cover the entire genome but lack quantitative and dynamic information.

In a new study, Song Li, Sarah M. Assmann, and Réka Albert create a dynamic model, based on a wide range of experimental observations that describes a complex signaling network in plants that is initiated by a plant hormone called abscisic acid (ABA). They circumvent a lack of quantitative data, especially concerning interaction strengths and reaction rates, of components in ABA signaling by using a computational technique that implicitly incorporates a range of possible quantitative parameters. Their model describes the regulation of over 40 network

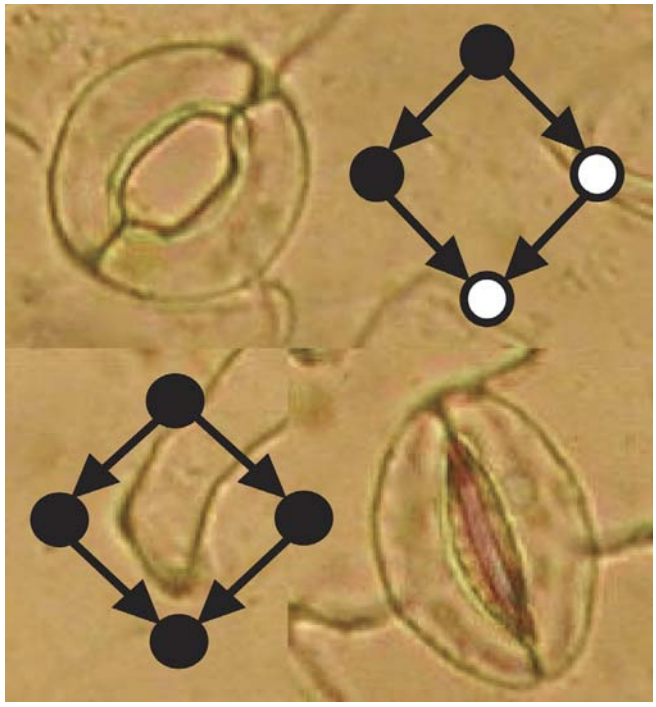
components, demonstrates the network's response to a range of perturbations through simulations, and makes novel, testable predictions about the sensitivity of the signaling pathway.

Plant growth and survival depend on the regulation of stomatal pores, which allow both carbon dioxide uptake for photosynthesis and water release through evapotranspiration. Signaling pathways triggered by ABA (a stress hormone secreted by the roots and synthesized by guard cells surrounding the pores), inhibit stomatal opening and promote closure, allowing the plant to conserve water during drought. ABA signaling triggers changes in cytosolic calcium through intermediate messengers that regulate the release of calcium from internal stores or the import of extracellular calcium; it also triggers the increase of cytosolic pH, and modulates a number of enzymes and cellular metabolites. As a result, membrane-localized ion channels open, releasing potassium ions and the negatively charged chloride and malate ions, leading to stomatal closure.

To shed light on ABA signaling dynamics, Li et al. synthesized published experimental data, mostly from *Arabidopsis thaliana* studies, about the components and processes of ABA signaling into a theoretical network. Experimental evidence described either direct interactions between components (from biochemical data on enzymatic activity or protein–protein interactions) or inferences about pathway activity (from genetic mutations or pharmacological interventions). These annotated components formed nodes (and intermediate nodes, representing unknown mediators) in the network, and the annotated processes provided the basis for writing algorithms describing possible interactions between the components and constructing the paths of the network.

Li et al. determined all the paths that nodes participated in and simulated experimental perturbations to individual nodes to predict how the network structure responds to such disturbances. They found several independent paths between the ABA input and the stomatal closure output. For example, the path involving pH-induced anion efflux does not overlap with paths regulating calcium levels, which can in turn be elevated through several independent paths. This independence, the researchers argue, indicates a "remarkable topological resilience" in which functionally redundant paths maintain ABA sensitivity in the face of disruptions in other pathways.

The path analysis describes routes from input to output, but can't reveal synergism between nonoverlapping paths. To do this, Li et al. used a dynamic modeling technique based on binary (active/on or inactive/off) assumptions about the state of interacting nodes to predict the probability of stomatal closure. Stomata don't usually open or shut completely, however, but close to varying degrees. The researchers captured this individual variability in the model by measuring stomatal apertures in *Arabidopsis* plants in the presence or absence of ABA application. These experiments provided population-level data to set a threshold between the open and closed state. With scant data on interaction times, component decay rates and initial states, they randomly assigned these values to cover the range of possibilities in over 10,000 simulations. They found that ABA induces complete closure in eight time steps; without ABA, the probability of closure is zero by the sixth step.



DOI: 10.1371/journal.pbio.0040323.g001

The authors model guard cell opening (upper panel) and closing (lower panel) in response to the plant hormone abscisic acid. (Image: Song Li)

Systematically perturbing this dynamic model system—simulating the effects of knocking out a gene or pharmacologically inhibiting a protein's activity—identified three single disruptions that made the system insensitive to ABA: loss of membrane depolarizability (which would prevent potassium channels from opening), disruption of anion efflux, and loss of actin cytoskeleton reorganization. Simulating two or three perturbations at a time showed that most of these multiple disruptions resulted in reduced (but not completely lost) ABA sensitivity. Simulated perturbations also predicted the integral contribution of cytosolic pH increase to ABA signaling—which Li et al. confirmed by showing that experimentally clamping cellular pH levels blocks stomatal closure. These results suggest that experimentalists would do well to further explore the role of cytosolic pH in ABA signaling.

While the researchers acknowledge that their network reconstruction is incomplete, it will easily incorporate new nodes as new signaling components are discovered and become more robust as quantitative experimental data emerge. This study shows how mathematical modeling and theory can synthesize a body of incomplete information on signal transduction to make predictions about the relative importance and behavior of network components. And with these predictions, experimentalists can focus their investigations on the most promising avenues of inquiry to reveal fundamental insights into the dynamics of complex biological systems.

Li S, Assmann SM, Albert R (2006) Predicting essential components of signal transduction networks: A dynamic model of guard cell abscisic acid signaling. DOI: 10.1371/journal.pbio.0040312

The Case of XPD: Sometimes Two Different Mutant Genes Are Better than One

Liza Gross | DOI: 10.1371/journal.pbio.0040347

Rare inherited disorders have long provided a unique window on the genetic basis of disease. Individuals inherit two copies of a gene; one from each parent. In a recessive disorder, if one copy is defective (a mutant allele), the alternate copy is usually sufficient to maintain normal function of the encoded protein. If an individual inherits two defective copies, protein function is disrupted, leading to disease. Often, two different mutant alleles of the same gene are present in one person, a phenomenon called compound heterozygosity. Whether these still cause disease depends on the gene in question.

The potential for recessive genes to interact has rarely been studied in human disease largely because distinguishing the effects of environment and genetic background from "biallelic" effects is very difficult in humans. In a new study, Jaan-Olle Andressoo, James Mitchell, and colleagues circumvent this problem by using a compound heterozygous mouse model of a severe human syndrome called trichothiodystrophy (TTD) that allowed them to link physical traits (or phenotype) to specific combinations of mutant alleles.

TTD belongs to a class of rare, clinically distinct XPD-related recessive disorders. XPD, a DNA-unwinding enzyme, is essential for both gene transcription and DNA repair of sun-induced damage as a component of the transcription/repair factor IIH (TFIIH) complex. In addition to TTD, XPD mutations cause xeroderma pigmentosum (XP) and Cockayne syndrome (CS). XP results in dramatically elevated cancer risk from extreme sun sensitivity—though, surprisingly, sun sensitivity does not necessarily cause skin cancer—and, in severe cases, primary neurodegeneration. Neither CS nor TTD increase cancer risk but lead to accelerated aging, reduced stature, and degeneration of the nerves' protective myelin sheath. TTD also causes scaly skin and brittle hair. As its name implies, the even rarer XP combined with CS (XPCS) combines cancer predisposition with neurodevelopmental problems.

In the current model, which Andressoo et al. refer to as the "monoallelic" paradigm of XPD-related disease, "causative" mutations are linked to a specific XPD syndrome. Mutations that aren't linked to a particular disorder are considered biologically inactive, or null. But the appearance of patients with causative mutations and a rare combination of TTD and XP symptoms has revealed the limitations of this paradigm. To explore the potential role of what the researchers call "biallelic effects" in human recessive disorders, Andressoo et al. asked whether different allele combinations of the enzyme XPD influence the diverse phenotypes associated with XPD-related recessive disorders. They discovered that combinations of XPD recessive alleles produced a variety of biallelic effects, from alleviating the severity of various disease symptoms to improving the function of the interacting genes.

In addition to their existing TTD mouse model (with the causative XPD^{R722W} mutation), Andressoo et al. "knocked in" a mutation found in an XPCS "hemizygote" patient (XPD^{G602D}), who had only one copy of the gene, suggesting that mice carrying two copies of this allele (homozygotes) should live. However, no Xpd^{G602D} homozygous mutants lived, and the Xpd^{G602D} allele was designated $Xpd^{\dagger XPCS}$ or "lethal." Lethality was likely caused by the reduced expression of the mutant allele rather than by the mutation itself, the researchers concluded, because they found in a separate study that normalization of XPD^{G602D} expression levels leads to viable homozygous animals. Thus, the XPD^{G602D} protein is likely biologically active but its reduced expression in homozygous $Xpd^{\dagger XPCS}$ animals causes lethality. Knocking in an *Xpd* mutation (encoding XPD^{R683W}) associated with XP was also homozygous lethal (and designated $Xpd^{\dagger XP}$), probably for the same reason.

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The surprising rescue of disease symptoms in mice suggests new thinking about recessive disorders in humans. Potential phenotypic consequences of compound heterozygosity are represented by a continuum from dark (disease) to light (health). (Photo: L. Kallasvee/J.O. Andressoo; Model: Reet Aus)

To see if these homozygous lethal alleles might interact with a different disease-causing allele, the researchers generated compound heterozygous mice with the *Xpd* homozygous lethal allele ($Xpd^{\dagger XPCS}$) and a TTD-causing allele (Xpd^{TTD}). Multiple skin, hair, and aging-related features of TTD were far less severe in the compound heterozygous animals than in animals carrying two copies of the TTD-causing allele. Beyond ameliorating these classic TTD symptoms, the homozygous lethal allele alleviated anemia

and developmental delay and also extended lifespan in the compound heterozygotes. Similarly, generating compound heterozygotes from the homozygous lethal XP allele ($Xpd^{\dagger XP}$) and the TTD-causing allele attenuated the TTD-related skin and weight-loss symptoms. The researchers propose that, due to the low expression levels, the lethal alleles, when homozygous, lead to a transcriptional defect that proves fatal. But when either allele is combined with the TTD-causing allele, the latter steps in to perform the transcription task early enough to prevent embryonic lethality. Then, later, as the skin, hair, and blood cells develop, the lethal alleles recover the deficiencies of the TTD allele.

Combining one of the homozygous lethal alleles ($Xpd^{\dagger XPCS}$ or $Xpd^{\dagger XP}$) with a TTD-causing allele also allowed the normally sun-sensitive XPCS and XP cells to better survive ultraviolet light. This finding suggests that interactions between the alleles produce an effect—resistance to sunlight—that neither has on its own, a phenomenon called "interallelic complementation." The researchers suspect that complementation occurs as different XPD molecules are plugged into the TFIIH complex at the site of DNA damage.

These results suggest that even though presumed-"null" alleles can't execute their transcription task, they may still influence disease outcome in compound heterozygous patients, as they have in the mouse model. The evidence that both alleles can contribute to disease phenotype, the researchers conclude, also suggests that it's time to adopt a biallelic paradigm for compound heterozygous patients with XPD-related disorders.

Andressoo JO, Jans J, de Wit J, Coin F, Hoogstraten D, et al. (2006) Rescue of progeria in trichothiodystrophy by homozygous lethal *Xpd* alleles. DOI: 10.1371/journal.pbio.0040322

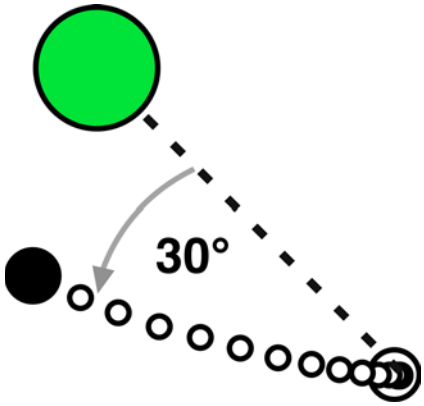
Learning New Movements Depends on the Statistics of Your Prior Actions

Mason Inman | DOI: 10.1371/journal.pbio.0040354

It's tough to learn to drive on the other side of the road than you're used to—just ask any American driving in London for the first time. Yet there's little you can do consciously to change such habits; you simply have to take the time to practice with the new set of rules. Now a new study shows that if people are given appropriate cues and learn tasks in a certain order, they can learn new rules more quickly and call them up at the right time.

John Krakauer and colleagues wanted to figure out how people learn new movements, like controlling a computer mouse, and then commit them to motor memory, to be reactivated when necessary. But learning can be a double-edged sword: sometimes learning one task makes it easier to learn another; other times the skills you've already picked up make it harder to learn something new.

It's been hard for researchers to understand when a person learning a new task will benefit from their past experience, and when history is a hindrance. It's also not clear what cues people might use to call up the appropriate set of rules from what they've learned before. This study shows that the benefit or hindrance afforded by training is itself dependent on the history of the learner, and that this history-dependent pattern obeys Bayesian statistics—which use prior knowledge to predict an outcome. Importantly, the statistics that matter seem to be the history of motion of various body parts.



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To study the effects of prior training on motor learning, the authors trained subjects to reach to an endpoint at a 30° angle from a visual target with either a wrist or an arm movement.

Krakauer and colleagues thought that perhaps people are unconsciously influenced by the history of how they have used their various body parts to learn a movement, and that this memory strongly influences how they will learn other new tasks. In effect, no learner is a blank sheet, but approaches every new task with strong biases. These biases are the accumulated history of how they have used their body in the past.

To test this possibility, they had people control a computer cursor using either just wrist movements or their whole arm and shoulder, with the wrist immobilized. Participants had to learn to adjust to having their control over the cursor manipulated: if they tried to move the cursor up, for example, it would move up and to the right; moving the cursor down would send it down and to the left. Krakauer and colleagues found that when a person learned to cope with one rotation first (with the arm), it helped them learn to cope more quickly with the same rotation with their wrist. But the reverse was not true: learning first with the hand did not aid learning with the arm. So, when learning new movements, the body faces the problem of deciding which body part to give credit for learning a task, the researchers argue. Since movements of the arm also include moving the wrist and hand with it, then learning with the arm usually affects learning with the wrist and hand, too. But during most wrist movements, the arm is relatively still, so learning at the wrist stays at the wrist.

Then, in more-complex experiments, the researchers showed they could block generalization from the arm to the wrist. In these experiments, they had people learn one rotation—say, a clockwise one—using their wrist, and then the opposite rotation (counter-clockwise) using their arm. This learning of a clockwise rotation with the wrist, then counter-clockwise with the arm, did not disrupt what had been already learned at the wrist because testing again with clockwise rotation with the wrist showed that people could call up their previous training—interference had been blocked. So people were able to acquire two opposite rules, as long as they learned them with different body parts.

In a similar test, people went through the same first two steps: clockwise rotation at the wrist, then counter-clockwise rotation with the arm. Then they tried to learn counter-clockwise rotation with the wrist—but they were no better than novices at this–transfer had been blocked. So both experiments showed that previous training at the wrist blocked the generalization of learning from arm to wrist that would otherwise have occurred. It seems that using different body parts to learn different rules is enough for people to keep the rules separate.

Krakauer and colleagues bolstered their experimental findings by comparing them with a statistical model of movements of the arm and wrist. The model uses a Bayesian approach, where previous experience and new data are combined to form a new parameter estimate. A key aspect of this approach is that greater uncertainty about the parameter leads to faster learning. Crucially, in their model, the investigators assumed that the majority of movements with the arm also include moving the wrist, but not vice versa. This led to a situation where the uncertainty in the parameter estimate—the imposed rotation—depended not only on the current limb context but also on the history of training in previous limb contexts. The model was able to reproduce most of the effects they saw in the experiments, such as the finding that learning would transfer from arm to wrist, but not vice versa, and blocking of generalization.

Together, these studies suggest that when learning two different rotations, learning the second rotation does not disrupt consolidation of the memory of the first, Krakauer and colleagues argue. Instead, the history of training with each body part and how the body parts work together determine whether a person can learn two different mappings and call them up at the appropriate times.

Krakauer JW, Mazzoni P, Ghazizadeh A, Ravindran R, Shadmehr R (2006) Generalization of motor learning depends on the history of prior action. DOI: 10.1371/journal.pbio.0040316

When Just One Sense Is Available, Multisensory Experience Fills in the Blanks

Liza Gross | DOI: 10.1371/journal.pbio.0040361

Our brains are wired in such a way that we can recognize a friend or loved one almost as easily whether we hear their voice or see their face. Specialized areas of the brain—in this case, the visual and auditory networks—are specially tuned to different properties of physical objects. These properties can be represented by multiple sensory modalities, so that a voice conveys nearly as much information about a person's identity as a face. This redundancy allows rapid, automatic recognition of multimodal stimuli. It may also underlie "unimodal" perception—hearing a voice on the phone, for example—by automatically reproducing cues that are usually provided by other senses. In this view, as you listen to the caller's voice, you imagine their face to try to identify the speaker. In a new study, Katharina von Kriegstein and Anne-Lise Giraud used functional magnetic resonance imaging (fMRI) to explore this possibility and understand how multimodal features like voices and faces are integrated in the human brain.

Studies using fMRI have established that when someone hears a familiar person's voice, an area of the temporal lobe called the fusiform face area (FFA) is activated through temporal voice areas (TVAs), suggesting early interactions between these cortical sensory areas. von Kriegstein and Giraud wondered whether these cortical ensembles might lay the foundation for general "multisensory representation" templates that enhance unimodal voice recognition.

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Recognizing people on the phone: Does knowing the face help? (Photo: copyright FEEI/FMK)

To explore this question, the researchers analyzed changes in brain activity and behavior after people learned to associate voices with an unfamiliar speaker. One group of participants learned to associate voices with faces and a second group learned to associate voices with names. Though both types of learning involve multimodal associations, voices and faces provide redundant information about a person's identity (such as gender and age), while voices and names provide arbitrary relationships (since any name could be associated with any voice). To further explore the contribution of redundant stimuli from the same source, the researchers added an additional set of conditions in which participants associated cellular phones with either ringtones or brand names. In this case, both cell phone and brand name were arbitrarily associated with a ringtone.

In the first phase of the fMRI experiment, participants listened to and identified either voices or ringtones. In the second phase, one group of participants learned to associate the voices and ringtones with faces and cell phones, while another group learned voice–name and ringtone–brand name associations. In the third phase, participants again heard only the auditory signals and identified either voices or ringtones as in phase one.

The brain scans showed that learning multisensory associations enhanced those brain regions involved in subsequent unimodal processing for both voice–face and voice–name association. But at the behavioral level, participants could recognize voices that they had paired with faces much more easily than they could recognize voices they had linked to names. Participants who had learned to associate voices with faces were the only ones to show increased FFA activation during unimodal voice recognition.

The fMRI results show that even a brief association between voices and

faces is enough to enhance functional connectivity between the TVA and FFA, which interact when a person recognizes a familiar voice. In contrast, voice–name association did not increase interactions between voice and written name sensory regions. Similarly, people did not recognize ringtones any better whether they had learned to associate them with cell phones or brand names. Nor did their brain scans reveal any interactions between auditory and visual areas during ringtone recognition.

Altogether, these results show that learning voice–face associations generates a multimodal sensory representation that involves increased functional connectivity between auditory (TVA) and visual (FFA) regions in the brain and improves unimodal voice recognition performance. When only one sensory modality of a stimulus is available, the researchers conclude, one can optimally identify a natural object by automatically tapping into multisensory representations in the brain—cross-modal ensembles that are normally coactivated—as long as the stimulus provides redundant information about the object. Given that faces and voices are the major means of social communication for nonhuman primates as well as for humans, the reliance of multiple, redundant sensory modalities likely has deep roots in our evolutionary history.

von Kriegstein K, Giraud AL (2006) Implicit multisensory associations influence voice recognition. DOI: 10.1371/journal.pbio.0040326

Islands Spark Accelerated Evolution

Liza Gross | DOI: 10.1371/journal.pbio.0040334

The notion of islands as natural testbeds for evolutionary study is nearly as old as the theory of evolution itself. The restricted scale, isolation, and sharp boundaries of islands create unique selective pressures, often to dramatic effect. Following what's known as the "island rule," small animals evolve into outsize versions of their continental counterparts while large animals shrink. Once restricted to islands, small animals often lacked predators and the competition between species that constrained the growth of their relatives on the mainland. Large mammals, on the other hand, no longer had access to vast grasslands and other abundant food sources and grew smaller to survive. Giant tortoises and iguanas still inhabit the Galápagos and a few other remote islands today, but only fossils remain of the dwarf hippopotami, elephants, and deer that once lived on islands in Indonesia, the Mediterranean, and the Pacific Ocean.

The fossil record suggests that these size changes (as well as other morphological changes) occur rapidly after species become isolated on islands, but this assumption has never been empirically examined in a systematic manner. In a new study, Virginie Millien puts this longstanding hypothesis to the test by analyzing the fossil record and data from living species. Comparing the rates of evolutionary change between island and mainland populations for 88 species at intervals ranging from 21 years to 12 million years, Millien confirmed that island species undergo accelerated evolutionary changes over relatively short time frames, between decades and several thousand years. (One can imagine rats of horror movie proportions if such rates persisted for millions of years.)

Measuring rates of evolutionary change has proven difficult because the fossil record rarely captures every morphological shift in a lineage, precise dating isn't always possible, and it's often not clear when the ancestral form first appeared on the island. To get a robust sample of island and mainland mammalian species, Millien collected data from text, figures, and tables in an extensive survey of the published literature. From these datasets, she calculated a total of 826 evolutionary rates for 170 populations representing the 88 species. (Rates of evolutionary change are measured in units called, appropriately enough, "darwins.")

Evolution rates, she found, decreased over time intervals for both island and mainland species, with a slower rate of decrease for island species. The differences in evolutionary rates between island and mainland pairs also decreased over time, becoming statistically insignificant for intervals over 45,000 years. Overall, island species evolved faster than mainland species—a phenomenon that was most pronounced for intervals between 21 years through 20,000 years.

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Giant tortoises at the Darwin Station on Isla Santa Cruz in the Galápagos Islands. (Photo: Catriona MacCallum)

Island evolution theory predicts that the most extreme effects of isolation will be seen on the smallest, most far-flung islands. In keeping with theory, Millien found that evolutionary rates for different populations of the same species varied with island locale. Thus, the rate of evolution does not appear to be an evolutionarily conserved trait, like metabolic rate or whiskers.

Because rodents make up nearly half of the world's mammalian species—and over 70% of taxa on some islands in this study—Millien repeated her analysis on a subset of the data with equal numbers of rodent and non-rodent taxa. The overrepresentation of rodents had no effect on the results, which still revealed significant differences between island and mainland evolution rates for the same species or populations.

The finding that mammals evolve faster on islands, Millien argues, comports with the island evolution theory prediction that mammals respond to their new island homes with rapid morphological and size adaptations. The brisk pace of these changes may explain why the fossil record harbors few examples of intermediate forms between the mainland ancestor and island descendant. Millien's results also conform with the hypothesis that evolution rates for island species slow down after the initial period of accelerated change, approaching rates on the mainland.

If island species can evolve quickly, Millien argues, it stands to reason that mainland species retain a similar capacity. As habitat destruction continues to pose the number one threat to biodiversity, many mainland habitats are beginning to resemble islands, with isolated pockets of wildlife separated by degraded or developed lands. Thus, island species may serve as a model for understanding how mainland species will adapt to the rapidly changing environmental conditions brought on by habitat destruction and global warming. It appears that some mainland species are already responding like island species: a 1989 study followed the island rule in linking fragmented habitat to body size changes in 25 European mammals over the past 200 years. How long animals can continue to evolve in the face of these changes, however, remains to be seen.

Millien V (2006) Morphological evolution is accelerated among island mammals. DOI: 10.1371/journal.pbio.0040321