Research Digest

Synopses of Research Articles

Evolution of a Primate Defense against Intragenomic Infiltrators

DOI: 10.1371/journal.pbio.0020292

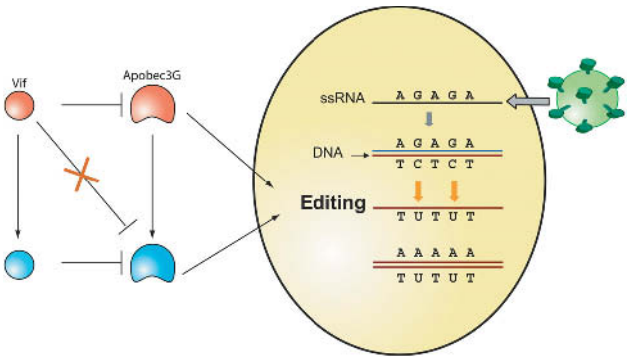
Anyone who uses a word processor is likely thankful for the spell checker program. But that autocorrect function can introduce errors, "correcting" the spelling of words to fit its stored repertoire, which is decidedly limited. Take that one step further and imagine a rogue program that destroys the coherence and meaning of your prose by swapping out one letter for another throughout the document. That's the situation retroviruses like the human immunodeficiency virus (HIV) face during the course of their infectious cycle, when a protein encoded by the host genome slips into the virus, mutates the virus's genetic material, and alters the viral genome.

The gene, *APOBEC3G*, belongs to a family of primate genes that produce enzymes (in this case, APOBEC3G) that "edit" DNA and RNA, by slipping into viral particles and inducing mutations that replace one base (cytosine) with another (uracil) as the virus undergoes reverse transcription in the host cell's cytoplasm. The edited virus fails to replicate. HIV, in turn, generates a protein called Vif that binds to the APOBEC3G enzyme and targets it for degradation, thereby eliminating its antiviral activity.

Since the protein-binding regions that govern these interactions have a direct effect on the fitness of both virus and host, one would expect to see the proteins angling for advantage, with Vif maximizing its ability to recognize APOBEC3G and APOBEC3G doing its best to evade Vif. Such battles are thought to result in frequent mutations that alter the amino acids involved in the interaction; the perpetuation of such advantageous mutations is called positive selection.

In this issue of *PLoS Biology*, Sara Sawyer, Michael Emerman, and Harmit Malik investigate the genetic roots of this battle for evolutionary advantage and find something surprising. As predicted, the *APOBEC3G* gene is under strong positive selection. But that selection appears to predate the existence of HIV-type viruses.

To characterize the selective pressures on *APOBEC3G* evolution, Sawyer et al. analyzed the gene from twelve primates—New World monkeys, Old World monkeys, and great apes, including humans—spanning 33 million years of evolution. Most of the primate lineages showed evidence of positive selection, indicating that the gene has been under pressure to adapt throughout the history of primate evolution. But viruses like HIV have been found in only five of the primates studied—three African monkeys, chimpanzees, and humans—and appear to be at most one million years old. And HIV infection in human populations is too recent to account for the positive selection of *APOBEC3G* in humans—so what has been fueling *APOBEC3G*'s rapid evolution?



10.1371/journal.pbio.0020292.g001

Genetic conflict between the host antiviral editing enzyme APOBEC3G, and the viral Vif protein leads to rapid fixation of amino acid replacements in both proteins

APOBEC3G and Vif interact in T-cells, but the fact that selective pressure on *APOBEC3G* has been constant over the course of primate evolution suggests that another force is also acting on the gene. Sawyer et al. propose that this force is most likely occurring in germline cells (sperm and egg precursors), which also produce high levels of *APOBEC3G* and can pass mobile genetic elements on to the next generation. Despite being non-infectious, these elements increase their own copy number in the host genome, moving from one part of the genome to another. The human genome is littered with such "retrotransposons," and it is these mobile genetic elements, the authors conclude, that likely antagonize *APOBEC3G*.

One class of retrotransposons, called human endogenous retroviruses, acts in many ways like foreign retroviruses. A retrovirus emanating from one's own genome poses less of an immediate threat than a retrovirus like HIV. But the constant efforts of the endogenous retrovirus to "jockey for evolutionary dominance," the authors conclude, could eventually take a toll and would be expected to provoke efforts to contain it. And it may be that this ancient intragenomic conflict endowed APOBEC3G with the means to do battle with foreign retroviruses like HIV.

Sawyer et al. also found evidence that five other *APOBEC* human genes appear to be engaged in similar conflicts. Combined with the finding that rodents have only one *APOBEC3G* gene and that five out of the six human *APOBEC3* genes have been under positive selection, these results suggest that this gene family expanded in mammalian evolution as a means of defending the germline from the promiscuous intrusions of mobile genetic elements.

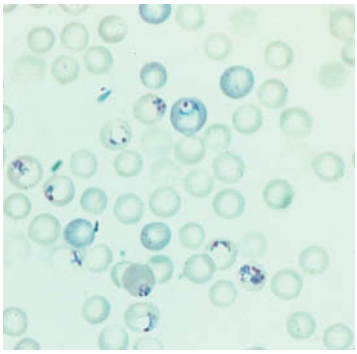
Sawyer SL, Emerman M, Malik HS (2004) Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. DOI: 10.1371/journal.pbio.0020275

Host Immunity Escalates the Evolution of Parasite Virulence

DOI: 10.1371/journal.pbio.0020251

Strictly defined, evolution is a change in the gene pool, or total set of genes, of a given population over time. Genetic changes that increase the fitness of an organism—that is, increase survival or fertility—are more likely to be retained, through natural selection, and passed on to succeeding generations. In the classic case of Darwin's finches, different ecological niches exerted different selective pressures on an original population, and resulted in 14 different species, each sporting a beak uniquely adapted to harvesting particular available food sources.

When it comes to microbial evolution, an ecological niche often takes the form of a host. If the microbe is a pathogen, its presence might trigger strong selective pressure from the host's immune system, precipitating an evolutionary two-step between microbe and host. Hosts with strong immune defenses can typically tolerate relatively virulent pests: conversely, ill-defended hosts die, which is bad news for the parasite. When the myxoma virus first infected a population of European rabbits in Australia in 1950, the virus was particularly lethal. Over time, less virulent strains were selected for—killing off your habitat would be an unsustainable fitness cost by most standards—and the rabbits developed resistance.



DOI: 10.1371/journal.pbio.0020251.g001

Malaria infecting red blood cells

In keeping with evolutionary theory, host immunity should affect the evolution of parasite virulence. Though theory predicts that immunity could potentially heighten virulence, there's no evidence that this is true. Being able to predict how natural selection will act on, and thus shape, virulence is vital for developing effective public health policies—and desperately needed vaccines—to deal with the ever growing roster of rapidly evolving pathogenic threats.

To investigate whether immune system defenses escalate pathogen virulence, Margaret Mackinnon and Andrew Read studied the malarial parasite *Plasmodium* in the mouse. Mackinnon and Read first directly injected two groups of mice with infectious parasites: "immunized" mice, which had been exposed to *Plasmodium* and then treated with the antimalarial drug mefloquine, and "naïve" mice, which had not. Parasites were serially transferred twenty times via a syringe from one mouse host to another. The virulence and infectiousness of the respective strains were evaluated by introducing the strains into another set of immunized and naïve mice.

As theoretically predicted, parasites evolved in the immunized mice were indeed more virulent than parasites evolved in the naïve mice. But what if the parasites were first transmitted through their natural vector, the mosquito, rather than through a syringe? Would they be as virulent? Interestingly, infection was not as severe after mosquito transmission. But parasites evolved in the immunized mice retained a higher level of virulence than those evolved in the naïve mice. This means that immunity accelerates the evolution of virulence in malaria, even after mosquito transmission, making them more dangerous to nonimmunized hosts.

How does immune selection create more virulent pathogens? One possibility is that even though many parasites die in immunized hosts, those that "win the race to the syringe"—or the mosquito—are likely genetically equipped to stay ahead of advancing immune system defenses.

It's not entirely clear why selection would favor more virulent parasites, but since the virulent strains showed no problems transmitting infection to new hosts, it's likely that such strains would spread throughout an immunized population. While mosquito transmission likely plays a significant role in virulence evolution—it clearly reduced virulence here—the molecular mechanics of this effect are also mostly speculative at this point. Many questions remain, but these results make a strong case that vaccine development aimed at protecting individuals against infectious pathogens would do well to consider the evolutionary implications, or increased pathogen virulence could be an unintended consequence.

Mackinnon MJ, Read AF (2004) Immunity promotes virulence evolution in a malaria model. DOI: 10.1371/journal.pbio.0020230

Fly Fights with Both Hands

DOI: 10.1371/journal.pbio.0020313

Defending against attack is one of the most important challenges facing any organism. But while sticks and stones may break the bones of a lion, microscopic threats such as bacteria require different weapons. And it's not just we humans who have this problem—insects are prey to bacterial infections too. Their immune systems, however, rely on a far simpler set of defenses than those found in mammals. Exactly how one insect immune system recognizes bacteria, and how it fights off the invader, is the subject of a new study in this issue by Johann Deisenhofer and colleagues.

The fruitfly, *Drosophila*, has long been known to use a set of molecular sentries called "peptidoglycan recognition proteins," or PGRPs, that circulate in the fly's bloodstream. When a PGRP recognizes a bacterial invader, it triggers a cascade of events whose ultimate product is a group of antimicrobial compounds that attack and kill the bacteria.

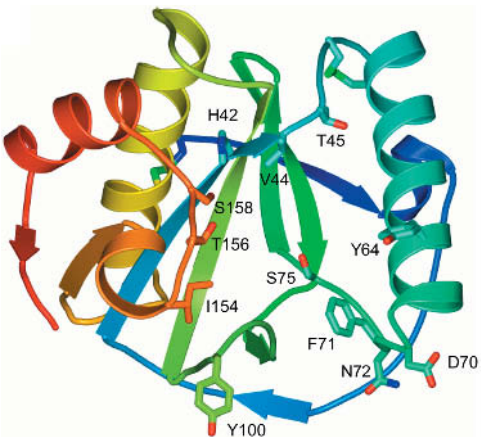
While the family of PGRPs has been extensively studied, exactly how they recognize their target bacteria has been less clear. At the cellular level, recognition requires contact, and the part of the bacterium the PGRP recognizes is, as its name implies, the peptidoglycan. A peptidoglycan is a special sort of molecular polymer found primarily on bacterial cell walls. Peptidoglycan forms when chains of sugar molecules (the glycans) are cross-linked by amino acids (the peptides) to form a meshwork that helps keep the bacterium from bursting under the osmotic strain of its contents.

There are several types of peptidoglycans that differ in their precise sugar and amino acid constituents and in their ability to trigger the *Drosophila* defensive reaction. Deisenhofer and colleagues set out to determine whether this difference in triggering ability of particular peptidoglycans was linked to differences in the PGRPs that recognize them. To do this, they determined the three-dimensional structure of one PGRP, called PGRP-SA. They worked out not only the overall shape of PGRP-SA, but also which amino acids sat where on the convoluted surface of the protein.

What they found on that surface was an extended groove down one entire side of the protein. To test whether this

groove was indeed the recognition site for peptidoglycan, the group introduced a series of mutations to critical amino acids along the groove, testing each new form for its ability to bind peptidoglycan. Indeed, the binding and defense-triggering ability was worse for almost every mutant, demonstrating conclusively that the normal protein uses the groove to bind and recognize peptidoglycan.

Then the team made a surprising discovery. They found that when PGRP-SA comes in contact with bacterial peptidoglycan, it begins to cleave the links between amino acids in the peptide portion of the peptidoglycan. This in itself is not so amazing—animals make plenty of peptide-cleaving proteins. But this protein has a difference, one which makes it unique in the animal kingdom.



DOI: 10.1371/journal.pbio.00020313.g001

Stick model of the PGRP-SA residues chosen for mutational analysis

To understand this difference, consider your two hands. They are mirror images of each other, alike yet not the same. No amount of twisting and turning will allow you to superimpose one exactly on the other—if you align the fingers, the knuckles will point in opposite directions, and if the knuckles point the same way, the fingers are all mismatched. This type of relationship between mirror images, called chirality (from the Greek for "hand"), is found in amino acids as well, a result of the three-dimensional geometry that radiates from their central atom.

All the amino acids used by all known animal species are exclusively of the "left-handed" form, and the protein-digesting enzymes we make are designed specifically for these L-amino acids. Bacteria, however, link left-handed and right-handed amino acids together to form peptidoglycan. What Deisenhofer's team discovered was that unlike any other known animal enzyme, the *Drosophila* PGRP-SA was able to break apart this "L,D" (levo-dextro) linkage, making it, in their words, "the first eukaryotic protein exhibiting such an activity specific for peptide bonds existing only in prokaryotes."

What does it all mean? Deisenhofer and colleages' results are yet another demonstration that at the molecular level, understanding structure is the key to understanding function. They also show that when it comes to defense, it helps to be able to fight with both hands.

Chang CI, Pili-Floury S, Hervé M, Parquet C, Chelliah Y, et al. (2004) A *Drosophila* pattern recognition receptor contains a peptidoglycan docking groove and unusual L,D-carboxypeptidase activity. DOI: 10.1371/journal.pbio.0020277

The Structural Basis of a Prostate Cancer Protein's Unique Selectivity

DOI: 10.1371/journal.pbio.0020305

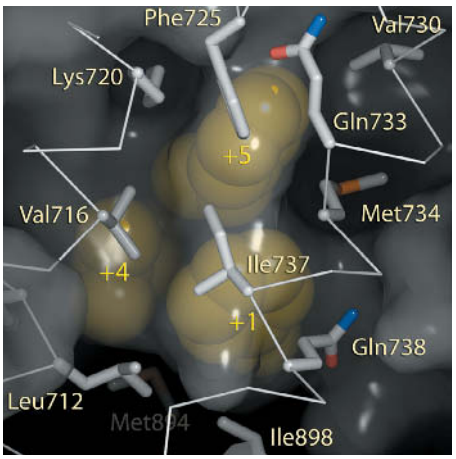
One of the major players in prostate cancer is a nuclear signaling protein called the androgen receptor. Prostate growth and development is regulated by androgen hormones (like testosterone) that activate the androgen receptor. When an androgen binds the receptor, the receptor binds other proteins, called coactivators, to activate genes controlling cell growth, survival, and differentiation. Unlike other receptors that function in the nucleus, the androgen receptor normally shuns coactivators with a leucine-rich binding domain in favor of those with "aromatic" domains. (Aromatic amino acids are defined by their ring structure.) But during prostate cancer, the receptor interacts with both coactivator types, to promote disease progression.

The secret to a protein's binding preference rests in the underlying sequence of its amino acids, which determines the protein's structure and ultimate behavior. Robert Fletterick and colleagues set out to identify the "full repertoire" of amino acid sequences that might conceivably consort with the androgen receptor. Their findings help explain the unusual behavior of the androgen receptor during prostate cancer progression—a first step toward developing new anticancer therapies.

Treatment for hormone-dependent prostate cancers focuses primarily on reducing androgen levels by using chemicals that compete for androgen receptor docking rights in the hormone-binding pocket of the ligand-binding domain, or LBD. (A ligand is a molecule, like the androgen hormone, that binds to a receptor.) But cancer cells eventually circumvent these chemical assaults through increased levels of either androgen receptors or their coactivators, or through mutations that make androgen receptors immune to chemotherapy. That's why Fletterick and colleagues turned their attentions to the receptor's consorts. Since targeting the hormone-binding pocket of the receptor offers limited benefits, a better strategy might involve disrupting associations with the receptor's coactivators.

Dozens of proteins interact with different regions of the androgen receptor, but the details of these interactions were not known. When a hormone binds to the LBD of other nuclear receptors, it triggers a conformational change that creates a binding surface called AF-2 for the leucine-rich domains of the coactivator proteins. It was not clear, however, how the AF-2 region of the androgen receptor distinguishes between aromatic and leucine-rich domains. To characterize the receptor's binding selectivity, Fletterick and colleagues tested 20 billion peptides, or protein fragments, to see whether they interacted with the LBD region of a hormone-bound androgen receptor.

As expected, most of the peptides that associated with the LBD domain were aromatic. And they interacted with the same region that naturally occurring coactivators bind to. Next, Fletterick and colleagues determined the three-dimensional structure of both the receptor bound to just the androgen hormone and the androgen–receptor pair bound to a subset of seven peptides. The different structures showed that the androgen receptor uses a single surface to bind both leucine-rich and aromatic peptides; when the aromatic peptides have



DOI: 10.1371/journal.pbio.0020305.g001

Surface complimentarity of hydrophobic motifs

bulky appendages, the receptor's AF-2 domain reorganizes to accommodate them.

The various structures and binding affinities for the different receptor–peptide complexes described here show how the receptor can interact with a diverse array of proteins. The androgen receptor, unlike other nuclear receptors, has specific amino acid sequences that better support aromatic peptide binding. Interestingly, mutations in one of these amino acid sequences have been found in prostate cancer. Altogether, the authors conclude, the unique properties of the receptor's AF-2 surface make it "an attractive target for pharmaceutical design." Drugs that directly interfere with coactivator binding, they explain, are likely to inhibit androgen receptor activity. Here, the authors recommend novel sites on the receptor as promising targets for androgen-receptor-specific inhibitors.

Hur E, Pfaff SJ, Payne ES, Gron H, Buehrer BM, et al. (2004) Recognition and accommodation at the androgen receptor coactivator binding interface. DOI: 10.1371/journal.pbio.0020274

Mitochondrial Genes Cause Nuclear Mischief

DOI: 10.1371/journal.pbio.0020316

While the nucleus of a cell may be its command headquarters, mitochondria are equally vital—they are the power plants of the cell, and without them all cellular activity would quickly and irrevocably come to a halt. Testifying to their origins as once free-living bacteria, mitochondria have their own DNA, comprising 37 genes in humans on a single circular chromosome. Whether they invaded their ancestral hosts as parasites or were captured as subcellular collaborators, they have long since left their independent ways behind. Their meager complement of genes is far fewer than is needed to produce these complex organelles; it is clear from analyzing the nuclear genome that most of the mitochondria's presumed ancestral genes have been taken into the cell's nucleus, where they are under the strict control of their host.

The transplanted mitochondrial genes have been faithfully doing their job under new management since they were first appropriated, probably hundreds of millions of years ago. However, not all of their DNA descendants have continued to make themselves so useful. For, in addition to many of the mitochondria's original genes, the human genome houses over 200 mitochondrial genetic fragments, useless pieces of code whose only remaining function is to be replicated generation after generation.

Detritus from other sources is even more common within the genome, and most of it seems to be harmless. But in this issue, Ricchetti and colleagues show that mitochondrial fragments may not be quite so benign. They have continued to invade the human genome, even into the present day, and a large proportion of them take up residence within nuclear genes, possibly disrupting them and causing human diseases.

Scanning the entire human genome, Ricchetti and colleagues found a total of 211 nuclear sequences of mitochondrial origin (NUMTs). Of these, they selected 42, which appeared to be the most recent integrations, for detailed study. Only 14 of them were also found in DNA from our closest relatives, chimpanzees, indicating that the rest arose after the human–chimp split approximately 5 million years ago. While 35 of the 42 were found in all humans tested, the rest were not, suggesting a still more recent origin for these among human populations.

The authors also made two surprising discoveries about the location of these human-specific NUMTs. They were not evenly distributed across the entire genome; instead, for reasons that are unclear, there were a disproportionate number of them on two chromosomes—the Y chromosome, present only in males, and number 18. Furthermore, NUMTs were not randomly scattered among all the DNA of the chromosomes. Rather, they were much less likely to be found in non-coding "junk" DNA and much more likely to have inserted themselves within highly active genes. This phenomenon is likely to be related to the mechanism by which a NUMT enters the chromosome—it relies on the machinery that repairs breaks in the DNA, and these breaks are more common in genes that are frequently transcribed. Such insertions can cause disease, as shown by the recent discovery of a hemophilia patient with a NUMT interrupting his clotting factor gene.

Much remains to be learned about the functional and temporal dynamics of NUMT insertions, but their potential for harm suggests that many NUMTS, unlike much of the rest of the flotsam that litters our genome, may be selected against quickly. Combined with their differential distribution among human ethnic groups, this may make them valuable markers for tracking both long- and short-term trends in human evolution and migration.

Ricchetti M, Tekaia F, Dujon B (2004) Continued colonization of the human genome by mitochondrial DNA. DOI: 10.1371/journal.pbio.0020273

Genome-Wide Survey of Cohesin: A Molecular Guardian of Genomic Fidelity

DOI: 10.1371/journal.pbio.0020291

At a fundamental level, the continuity of life depends on cell division. Humans generate many millions of cells per second just to stay alive, with most cell types dividing and multiplying repeatedly during a lifetime. Details of cell division vary from cell to cell and organism to organism, but certain features are universal, including what is arguably a cell's most crucial task: the faithful duplication and segregation of its genetic material.

During mitosis, a cell copies its nuclear DNA, then splits into two identical daughter cells, a process that involves moving the replicated chromosomes (called sister chromatids) toward opposite ends of the cell. After chromosomes replicate, a protein complex called cohesin binds the sister chromatids together. Cohesion helps the cell distinguish between the copies, which in turn aids proper distribution. Improper sister chromatid segregation can yield an abnormal number of chromosomes (called aneuploidy) in the daughter cells, a condition associated with cancer. During meiosis—the cell division that produces egg and sperm cells—aneuploidy causes a number of congenital disorders, including Down's syndrome.

To end up in their appropriate positions, sister chromatids must establish attachments to tentacle-like protein polymers called spindle microtubules, which emanate from spindle poles at opposite ends of a cell. Cohesion between the chromatids makes these bipolar attachments possible and keeps sister chromatids from separating after they attach to the spindle. Cohesion occurs along the length of a chromosome and is particularly strong around centromeres, the pinched region of a chromosome. Centromeres, in turn, assemble another protein complex called the kinetochore, which mediates the attachment of chromosomes to spindle microtubules; together, they guide chromosomes to their respective destinations.

DOI: 10.1371/journal.pbio.0020291.g001

PeakFinder automates identification of peaks in ChIP data

Cohesin's binding locations were discovered by removing chromatin—the mass of DNA and proteins that forms chromosomes—from cells, and purifying the regions associated with cohesin. These studies looked at cohesin's binding distribution either genome-wide or at select regions of a few chromosomes. Here, two research groups use a similar approach to provide a broader picture in their analysis of cohesin binding in the budding yeast *Saccharomyces cerevisiae*, a favorite system for cell biologists. In the first paper, Jennifer Gerton and colleagues generated a map for the entire yeast genome of locations where cohesin binds to chromosomes during meiosis and mitosis. In the second paper, Paul Megee and colleagues found that centromeres attract large concentrations of cohesin to their flanks and that the assembly of these cohesin domains is mediated by centromere–kinetochore complexes.

Gerton's group reports that large regions surrounding centromeres have "intense" cohesin binding. These binding sites correlate with DNA base composition—DNA is composed of four chemical bases, or nucleotides, that are referred to as A, C, G, and T—showing a strong association with AT-rich regions. In meiotic chromosomes, cohesin binding sites are interspersed between the DNA double-strand breaks that initiate the exchange of genetic information characteristic of meiosis, perhaps keeping the chromatids attached without interfering with genetic recombination.

Most striking, the authors note, is the observation that cohesin binding changes according to the cell's gene transcription program. Cohesin prefers DNA that lies between active transcription zones and is unceremoniously displaced from regions where RNA transcripts are being made (a process called elongation). This suggests that elongation through a region and cohesion binding may be incompatible. These observations support previous work indicating that DNA sequences required for the replication and segregation of chromosomes must be protected from transcription to function properly. Whatever the explanation, this finding begs the question of how more complicated genomes can accommodate these two seemingly contradictory processes.

Megee's group investigated whether all yeast chromosomes have these large centromere-flanking cohesin regions and whether the centromeres and DNA sequences that surround them somehow facilitate the assembly of cohesin complexes. By removing centromeres and generating cells incapable of assembling kinetochores, the researchers show that the assembly of these cohesin regions is mediated solely by the centromere–kinetochore complex.

What's more, inserting centromeric DNA sequences in abnormal chromosomal locations produced new cohesin-assembling regions around these "neo" centromeres. The kinetochores' influence appears to stretch over tens of thousands of DNA bases and serves chromatid segregation in two crucial ways: by recruiting high levels of cohesin to centromeres' sides, which attaches chromatids to their bipolar spindles, and by attaching chromatids to microtubules, which provides their passage to the cell's opposite sides. The maintenance of genomic integrity, the authors conclude, likely relies on the coordination of these essential functions.

Glynn EF, Megee PC, Yu HG, Mistrot C, Unal E, et al. (2004) Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*. DOI: 10.1371/journal.pbio.0020259

Weber SA, Gerton JL, Polancic JE, DeRisi JL, Koshland D, et al. (2004) The kinetochore is an enhancer of pericentric cohesin binding. DOI: 10.1371/journal.pbio.0020260

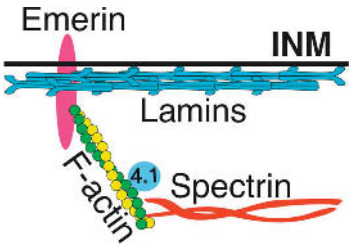
A Case for a Functional Actin Network in the Nucleus

DOI: 10.1371/journal.pbio.0020300

In June, muscular dystrophy patients lost one of their most passionate advocates to a rare form of this degenerative neuromuscular disorder—thirteen-year-old Mattie Stepanek. In his short life, Stepanek wrote five volumes of inspirational poetry, topping the *New York Times* bestseller list and winning accolades from the likes of Jimmy Carter. A wide range of inherited disorders falls under the rubric of muscular dystrophy, but all involve some form of progressive muscle wasting. Stepanek's condition impaired nearly all of his body's functions, but other more common forms, including Emery-Dreifuss muscular dystrophy (EDMD), selectively target skeletal muscle and induce cardiac abnormalities.

EDMD is caused by mutations in either of two genes: one encodes lamin A, a structural protein associated with the nucleus, and the other encodes a nuclear membrane protein called emerin. Lamins, a major component of the structural network that supports the nuclear envelope, help the nuclear envelope maintain structural integrity and absorb mechanical stress without rupturing. (Structures that support the nucleus and regulate molecular traffic between the cytoplasm and nucleus are collectively referred to as the nuclear envelope. They include the inner and outer nuclear membranes, the nuclear pore complexes, and a network of lamin filaments, called the nuclear lamina, near the inner membrane.) Emerin binds to proteins that regulate gene transcription. Emerin and lamins are found in most cell types, yet EDMD attacks only skeletal muscles, major tendons, and the cells that regulate cardiac muscle contraction. So where does this tissue specificity come from?

One theory suggests that emerin selectively targets proteins that specifically regulate gene expression in EDMD-affected tissues. Another theory proposes that emerin provides structural support to the nuclear envelope and that emerin mutations are most destructive in tissues subjected to mechanical stress—like skeletal muscle and tendons. Current evidence supports both models. Recent studies suggest that emerin forms complexes with actin—the mother of all structural proteins. Actin proteins can join together (polymerize) to form a variety of filaments. However, given longstanding doubts that actin exists in the nucleus, let alone functions there, researchers were unsure what the findings might indicate. Now James Holaska, Amy Kowalski, and Katherine Wilson propose that emerin not only functions as a structural protein in the nucleus but that it does so by interacting with actin.



DOI: 10.1371/journal.pbio.0020300.g001

Interactions of structural proteins at the nuclear membrane

Evidence that emerin and lamin A can form multiprotein complexes comes primarily from experiments in test tubes. To get a sense of the physiological significance of these findings, Wilson and colleagues purified emerin-binding proteins from the nuclei of living cells. They found that emerin binds to polymerized actin and, in fact, appears to stimulate polymerization. By binding and "capping" a specific end of the actin filament, emerin prevents filament de-polymerization (disassembly), effectively increasing the rate of actin polymerization by four- to twelve-fold. The authors propose that emerin "promotes the formation of a nuclear actin cortical network," which could serve to anchor membrane proteins and lamin filaments to the inner nuclear membrane and thus enhance the structural integrity of the nuclear envelope. Whether emerin also interconnects the lamin and actin filament networks at the nuclear envelope—which could significantly reinforce its mechanical strength—will have to await further study.

Muscle contraction places enormous stress on cell membranes. These results suggest that actin-based networks, in addition to lamin networks, support the structural integrity of the nuclear envelope. Defects in proteins involved in either network could compromise nuclear structure, which could in turn disrupt the cell's gene expression program, for example, or rupture the cell membrane, killing the cell. Subtle defects in proteins important for muscle cell integrity can cause several forms of muscular dystrophy. Now it appears that emerin defects could cause EDMD in part by compromising the mechanical integrity of nuclei in muscle cells and tendons.

Holaska JM, Kowalski AK, Wilson KL (2004) Emerin caps the pointed end of actin filaments: Evidence for an actin cortical network at the nuclear inner membrane. DOI: 10.1371/journal.pbio.0020231

Protein Helps Orchestrate Cells' Fluid Uptake

DOI: 10.1371/journal.pbio.0020318

You are what you eat and drink. Steak can sit in your stomach or orange juice wind through your intestines, but they only become part of your body once they're taken up by your cells. First, foods must be reduced to a soup of proteins, fats, sugars, and so on. But even then, getting these materials into a cell isn't as simple as sticking them in your mouth. For one, there's the membrane enclosing a cell. Simply puncturing a hole in the membrane would spill the cell's contents, harming or killing the cell.

Instead, all eukaryotes—organisms whose cells have nuclei—use a carefully orchestrated process called endocytosis to bring materials into their cells. Eukaryotic cells first form cavities in their cell membrane that surround nearby particles or fluid. These pockets seal shut and bud off into the cell to form small membrane-bound sacs called vesicles.

When taking in fluids, eukaryotic cells use two distinct mechanisms—to take tiny sips or huge gulps. With one process, called pinocytosis, cells continually form small pockets in the cell membrane that enclose small droplets of fluid in vesicles called pinosomes. These newly formed vesicles, called early endosomes, bud off from the membrane and fuse with other early endosomes. In one form of pinocytosis, the vesicles are encaged by a protein called clathrin that tightly constrains their size. These carriers incorporate membrane constituents (for example, growth factors) with very high selectivity. In macropinocytosis, on the other hand, large ruffles in the membrane engulf mass quantities of fluid in vesicles known as macropinosomes.

Beyond taking in nutrients, these processes are essential to the function of many organs—from the brain, where nerve cells receive other cells' chemical signals by pinocytosis, to the kidney, where cells use macropinocytosis to

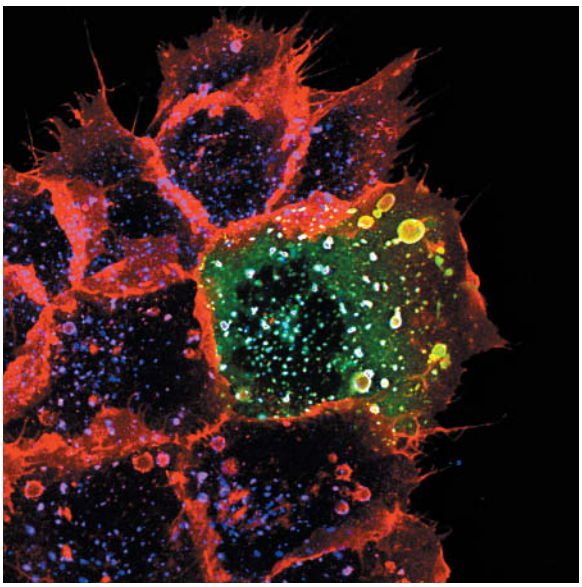
take in waste fluids for processing. Macropinocytosis is also relevant to cancer cells; it has long been known that oncogenes dramatically induce this endocytic process, affecting the signaling status of these cells. But compared with other types of endocytosis, molecular biologists know surprisingly little of the mechanisms behind macropinocytosis. They do know that the Rab5 protein—an enzyme that coordinates a complex network of other proteins, called effectors—is crucial for both pinocytosis and macropinocytosis.

Now, as reported in this issue of *PLoS Biology*, Marino Zerial and colleagues have found a new protein, which they named Rabankyrin-5, that forms a further link between these two mechanisms for fluid uptake. The protein is necessary for macropinocytosis, and its levels control the rate of this process. In addition, Rabankyrin-5 helps regulate endosome trafficking and coordinates this mechanism with macropinocytosis.

In two commonly used human and mouse cell lines, the researchers found the protein Rabankyrin-5 along with Rab5 on both types of pinosomes, early endosomes and macropinosomes. The early endosomes usually fuse with one another inside the cell, but when the researchers blocked Rabankyrin-5 activity, this fusion fell sharply. Suppressing Rabankyrin-5 activity also stifled macropinocytosis; overexpressing the effector, on the other hand, sent macropinocytosis into overdrive.

The researchers also looked at endocytosis in mouse kidney and canine kidney cell lines. Inside the kidney, fluid-carrying ducts are lined with epithelial cells that take up liquids through their exposed surface. The researchers found Rabankyrin-5 predominately on vesicles at this surface, and as in the other experiments, overexpression of the protein promoted macropinocytosis. Together, these findings suggest Rabankyrin-5 plays a role in regulating this form of fluid uptake and plays a role in kidney function. The discovery of Rabankyrin-5 involvement in macropinocytosis also has implications for other physiological and pathological mechanisms such as the immune system response, defense against pathogens, and hyperactivation of signaling pathways in cancer cells.

Rabankyrin-5 contains various regions that bind other proteins and also lipids found in cell membranes, suggesting the protein plays a mechanical role in forming vesicles. The protein also has regions found on other proteins that are involved in signaling and development, so it may help direct vesicles' traffic within the cell. The protein also has regions characteristic of proteins involved in clathrin-dependent endocytosis, which fits with the researchers' finding that Rabankyrin-5 affects pinocytosis.



10.1371/journal.pbio.0020318.g001

Rabankyrin-5 (green) colocalizes with rhodamine-conjugated EGF on macropinosomes after growth factor stimulation

All told, Rabankyrin-5 appears to form a bridge between two distinct mechanisms, pinocytosis and macropinocytosis, that cells use to take in fluids. While the details of how Rabankyrin-5 functions are still unclear, these findings give researchers a new handle for grasping how macropinocytosis works and how cells control when and how much they drink in their surroundings.

Schnatwinkel C, Christoforidis S, Lindsay MR, Uttenweiler-Joseph S, Wilm M, et al. (2004) The Rab5 effector rabankyrin-5 regulates and coordinates different endocytic mechanisms. DOI: 10.1371/journal.pbio.0020261

New Route to Longer Life

DOI: 10.1371/journal.pbio.0020308

Ever since the early Greeks recast humans as the center of the universe and remade God in their own image, Western philosophers and poets have grappled with the limits of human mortality. Philosophers found relief from Keats's "unwilling sleep" by dividing human existence into body and soul and asserting that the true essence of humanity lies in the immortal soul, not in the body. Ironically, as this decidedly nonscientific subject has lost favor with modern-day philosophers, it has captured the imagination of scientists. But, for now at least, the interest is in prolonging life rather than escaping mortality.

Over the past twenty years, mounting evidence from a wide range of organisms indicates that a longer life awaits those who eat less. In yeast, calories can be restricted directly, by limiting yeast's glucose supply, or indirectly, by inhibiting yeast's ability to metabolize glucose. Either way, many studies have suggested that the increased longevity associated with calorie restriction is linked to increased activity of a gene called *SIR2*. Now, Brian Kennedy and colleagues show that calorie restriction and *SIR2* promote longevity through distinct genetic pathways—and that aging in yeast and higher organisms may be more similar than previously thought.

One of the causes of aging in yeast is the accumulation of coiled bits of DNA, called extrachromosomal ribosomal DNA circles (ERCs), in the nucleus of a mother cell (which divides to create two identical daughter cells). An overabundance of these rDNA circles wreaks havoc on a cell and eventually kills it. Genetic mutations that reduce their levels are linked to increased life span. Mutations that disrupt the *FOB1* gene, for example, dramatically reduce ERC levels and increase the reproductive life span of cells by 30%–40%. In contrast, mutations that disrupt *SIR2* increase ERC levels and cut life span in half, while increasing *SIR2* activity increases life span by 30%–40%.

In previous experiments, several groups have identified a link between calorie restriction, *SIR2*, and the accumulation of ERCs. The idea is that calorie restriction somehow activates the protein encoded by *SIR2*, which in turn decreases ERC accumulation. Now, Kennedy's team has found that

the combination of calorie restriction and *FOB1* mutation increases life span more than either approach does alone. This finding was unexpected because previous studies showed that combining increased *SIR2* activity with *FOB1* deletion mutations did not extend life span. If calorie restriction extends life through *SIR2*, then combining either caloric restriction or *SIR2* overexpression with *FOB1* mutations should produce the same result.

This contradiction raised the possibility that calorie restriction operates through another mechanism, independent of *SIR2*. In support of this view, caloric restriction enhances life span to a greater extent in *FOB1* mutants lacking *SIR2* than in *FOB1* mutants with an intact *SIR2* gene. This and other genetic experiments indicate that calorie restriction does not always work through *SIR2*.

That suggests, the authors explain, that calorie restriction functions either by regulating ERC levels or by some still unknown molecular pathway. They conclude that the enhanced longevity seen in calorie-restricted *FOB1* mutants is not related to ERCs, because these yeast strains already have low ERC levels. Since calorie restriction is the only demonstrated approach to increasing life span in a diverse range of organisms, including mammals, and since there's no evidence that ERCs affect the aging of any organism besides yeast, these results bode well for understanding how calorie restriction works in higher organisms. And the finding that calorie restriction and *SIR2* operate through genetically distinct pathways in yeast, the authors conclude, suggests that certain aspects of both pathways might have been conserved through evolution. Working out the details of these pathways in yeast is the first step toward understanding which, if any, of these components might enhance longevity in humans. Of course, as any student of Greek mythology knows, longevity without eternal youth comes with a price.

Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. DOI: 10.1371/journal.pbio.0020296

Verifying Sequences that Enhance Splicing

DOI: 10.1371/journal.pbio.0020323

Identifying the causative mutation for a disease can be the first step to a potential cure. This task is not always trivial. Often the initial strategy is to look for the variations within a mutated gene that alter its protein coding sequence, as these mutations often alter the gene's function. However, in a growing number of cases, the causative mutation is a "synonymous" mutation—a change in the coding sequence of a gene that doesn't change the sequence of the protein coded by the gene. This type of mutation may be responsible for Seckel syndrome, a human disease characterized by dwarfism. In Seckel syndrome, the mutation doesn't alter the protein sequence itself but instead results in the skipping over of a portion of the protein coding sequence (an exon), a process called altered splicing. The disease-causing potential of this type of splicing mutation has only recently gathered attention.

Splicing assembles the exons of a transcribed gene (the RNA copy) into the right order while removing the non-coding sequences of the RNA (introns). This highly regulated process is coordinated by a number of sequences within a gene, including splice sites that precede and follow the exon, as well as by exonic splicing enhancers (ESEs), which help recruit the factors (proteins) necessary to insure proper splicing. Although splice sites have optimal (consensus) sequences, there is some variability amongst individual splice site sequences that allows splicing to take place to a greater or lesser extent. ESEs facilitate splicing, especially when a gene's splice sites vary from the consensus sequence. Candidate ESEs have previously been identified based on their more frequent occurrence in exons that are adjacent to non-consensus splice sites.

In this issue of *PLoS Biology*, William Fairbrother et al. investigated the functionality of these putative ESEs. If they are functional, the authors reasoned, then mutations that disrupt them would be selected against—that is, these mutations would tend to be discarded—in the human genome.

To this end Fairbrother et al. developed a computational method, which they call VERIFY (for "variant elimination reinforces functionality"), to evaluate the selective pressure on ESEs. They took advantage of a public database of all single nucleotide polymorphisms (DNA changes at a single point) within the human genome and compared them to the chimpanzee genome; this allowed the authors to infer the identity of the ancestral gene (or allele). By determining which allele is ancestral and which is the variant, the researchers could then distinguish the mutations that created ESEs from mutations that disrupted ESEs.

Mutations that altered or disrupted ESEs were under-represented, leading the authors to conclude that predicted ESE sequences evolve under a more stringent level of selection than exonic sequences with no predicted ESEs. This selective pressure was greater for predicted ESEs located near the splice signals than for ESEs that were located within the exon. This result was consistent with experimental findings that ESE strength diminishes with distance from the splice site.

As more vertebrate genomes are sequenced and the public database of single nucleotide polymorphisms continues to grow, this type of computational method will become increasingly valuable. It can help confirm the functionality or role of candidate regulatory elements thought to control various aspects of gene expression, and in so doing, offer insights into the complex machinations required to maintain the healthy operation of the human genome.

Fairbrother WG, Holste D, Burge CB, Sharp PA (2004) Single nucleotide polymorphism–based validation of exonic splicing enhancers. DOI: 10.1371/journal.pbio.0020268

Dissecting the Transcriptional Control of Body Patterning

DOI: 10.1371/journal.pbio.0020319

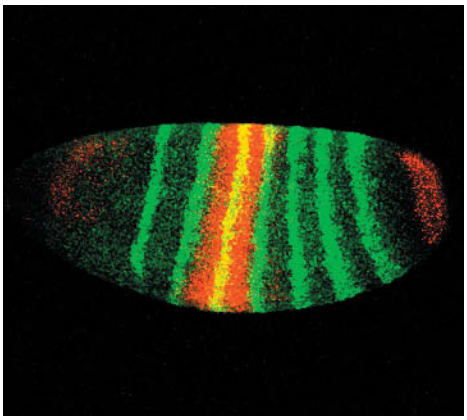
To build the complex body plan of higher organisms, thousands of genes must act in a coordinated fashion, becoming active at the right time and in the right place to define structures like head, thorax, and abdomen, or cell types like skin, muscle, and bone. One of the central questions for developmental biologists is how such specific spatiotemporal expression of genes is achieved.

The general mechanism of the control of gene expression is well understood: Special proteins, called transcription factors, bind to short stretches of DNA near a gene. By docking to such binding sites, they activate or repress the transcription of the gene into mRNA (which is then translated into protein). Transcription factors often act in a combinatorial fashion—that is, several different factors have to bind in close proximity to each other to achieve a particular transcriptional outcome. As a consequence, their binding sites form clusters, called regulatory elements or modules.

In many contexts, the genes that are activated or repressed encode transcription factors themselves, forming a cascade of transcriptional control events. One such transcriptional control hierarchy is the segmentation gene network in the fruitfly *Drosophila*. Organized in four tiers and acting in combinatorial fashion, the segmentation genes lay out the anterior-posterior axis of the embryo. In a stepwise refinement of expression patterns, they translate broad, overlapping gradients formed by maternally provided transcription factors into a periodic pattern of 14 discrete stripes that prefigure the 14 segments of the larva. The segmentation gene network has long been one of the prime paradigms for studying transcriptional control, and many researchers have worked over the years to experimentally dissect the regulatory interactions within the hierarchy. For some of the most important genes, the regulatory elements driving their expression and the favored binding sites have been identified. Nevertheless, the picture of transcriptional regulation within the segmentation gene network has remained incomplete.

This is where the research reported by Mark Schroeder et al. comes in: With the sequence of entire genomes available, it's possible to use existing binding site information to computationally search the neighborhood of genes for regulatory elements. The difficulty here is that in higher organisms such as *Drosophila*, the binding sites are typically short and variable, and the search space is large; on the other hand, the fact that sites cluster—where transcription factors work in concert—aids the task.

To identify regulatory elements, the researchers developed an algorithm, named Ahab, that models the behavior of multiple transcription factors competing for binding sites and fine-tunes the search by detecting clusters of weak sites. Using this approach, Schroeder et al. identified 52 regulatory elements within the segmentation gene network, 32 of them novel. The authors tested a large number of the newly identified modules experimentally by placing them in front of reporter genes that reveal where the modules drive expression within the developing fly. They showed that almost all modules faithfully reproduce the expression pattern of the endogenous gene. To better understand the way segmentation gene modules function, the researchers then systematically analyzed their predicted binding site composition. They correlated the composition of modules with the expression they produce and with the distribution of the transcription factors that bind to them. They were thus able to glean basic composition rules and to derive the mode of action for most of the factors, that is, whether they act as activators or as repressors.



DOI: 10.1371/journal.pbio.00020319.g001

Segmentation in the early *Drosophila* embryo

Overall, Schroeder et al. show that a computational search can greatly reduce the experimental effort necessary for finding regulatory elements within the genomic sequence. Their study provides an example of how experimental and quantitative methods can be combined to achieve a more global analysis of the regulatory interactions within a transcriptional network.

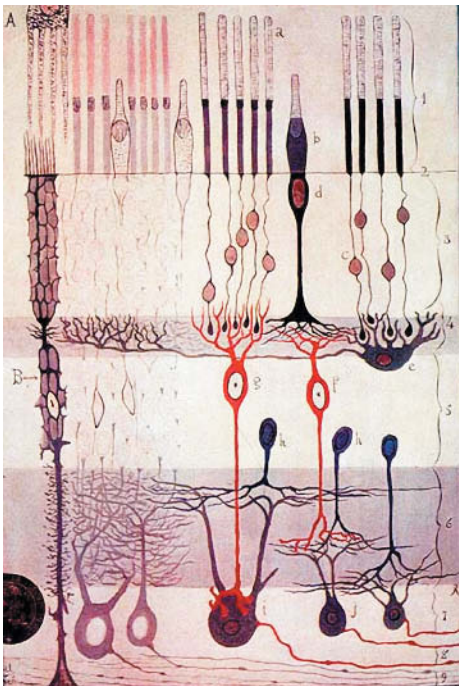
Schroeder MD, Pearce M, Fak J, Fan HQ, Unnerstall U, et al. (2004) Transcriptional control in the segmentation gene network of *Drosophila*. DOI: 10.1371/journal.pbio.0020271

Genomic Analysis of Retinal Development in the Mouse

DOI: 10.1371/journal.pbio.0020265

The eyes may be the window to the soul for poets, but for neuroscientists, they serve a more practical purpose. Of the 100 trillion or so cells that make up the human body, over 100 billion are dedicated to the structure and operation of the brain alone. Given the molecular and functional complexity inherent in such numbers, neuroscientists have historically focused on a more tractable system, the vertebrate retina, to study central nervous system development and physiology. Cells in the retina are packaged into highly ordered anatomical layers, based on their specialized functions. This organizational structure is characteristic of other regions of the central nervous system, and allows the brain to take in and integrate sensory information simultaneously, using discrete computational units. Creating such functional microprocessors depends on making the right cell at the right place and time.

During development, cells undergo periods of proliferation and increasing specialization (differentiation), generating seven types of retinal cells (six types of neurons and one glial cell type) in a precise order at specific times. Mature, specialized cells arise from a pool of proliferating progenitors—cells that have already



DOI: 10.1371/journal.pbio.0020265.g001

Classic drawing of the retina by Ramón y Cajal

committed to becoming a retinal cell but haven't yet settled on a particular cell type. But progenitors are not all alike; they display intrinsic differences in their "competence" to produce a particular subset of retinal cells at a particular stage of development. These differences may help ensure that ganglion cells, for example, are established before photoreceptors, since photoreceptors rely on ganglion cells to transmit their signals to the brain.

Which path a cell ultimately chooses stems from a combination of both intrinsic competence factors—likely determined by a cell's gene expression program—and external signals from the cell's environment. Progenitors give rise to "postmitotic" cells (cells that have exited the cell cycle and ceased proliferating), which go on to express characteristics associated with a specific cell type.

Beyond this framework, the molecular underpinnings of retinal development remain obscure. Differentiated cells exhibit a gene expression program unique to their cell type, but it's not clear what accounts for underlying differences among progenitors, for example, or what factors usher retinal cells into their respective specialties. To map the genetic landscape of retinal development, Constance Cepko and colleagues looked for genes expressed in retinal cells passing through various competence levels and making cell fate choices. They determined gene expression profiles by collecting bits of gene transcripts from the retinal tissue of developing mice at two-day intervals, starting with mice entering neurogenesis and ending with mice about six and a half days old. They also collected gene expression data from postnatal day 10 and from adult mice.

The authors then examined the cellular expression patterns of 1,051 of the genes that showed dynamic patterns by genomic expression profiling. Cepko and colleagues then pegged these genes to specific cell types to create a "molecular atlas of gene expression in the developing retina." (Though the retina has many millions of cells, different cell types can be easily identified based on their telltale shape and position in the retina.) Nearly every gene known to direct retinal cell differentiation was detected in this analysis and showed high levels of expression. Genes required for cell fate choices showed peak expression near or after cells exited the cell cycle, supporting the idea that similar controls operate to put the brakes on cell proliferation and to determine cell fate. Many uncharacterized genes were expressed only in certain progenitor subsets, making them good candidates as cell fate determinants for different subtypes of retinal cells. A promising list of candidate genes for retinal development and function appear in this molecular atlas, along with candidates for retinal disease. Since many degenerative retinal diseases stem from defects in development, these genes will help researchers focus their search for therapies. And if the eye truly is the window of the nervous system, these findings may suggest general principles of cell fate determination for the developing brain, spinal cord, and other regions of the vertebrate nervous system.

Blackshaw S, Harpavat S, Trimarchi J, Cai L, Huang H (2004) Genomic analysis of mouse retinal development. DOI: 10.1371/journal.pbio.0020247

Patterning the Face

DOI: 10.1371/journal.pbio.0020295

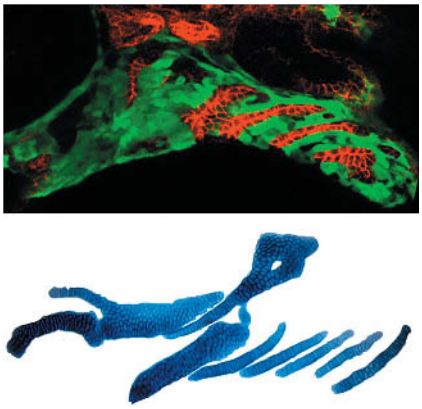
Vertebrates come in a dazzling array of shapes and sizes, from blue whales to pygmy bats, their overt morphology determined largely by the skeleton. The head skeleton in particular has undergone remarkable diversification, as is beautifully illustrated in Darwin's examination of beak morphology in Galapagos finches. It is now appreciated that a large part of the facial skeleton is derived from a newly identified, vertebrate-specific population of cells, called the cranial neural crest, that has its origins at the border of the dorsal neural plate (the future brain).

Vertebrates develop from three germ layers—the endoderm, mesoderm, and ectoderm—which each give rise to distinct elements in the emerging body plan, and interactions between these layers are a common feature of embryogenesis. For example, early in development, cranial neural crest cells migrate to positions along the bottom (ventral side) of the future head, where they form a series of developmental intermediate structures called pharyngeal arches. The arches facilitate interactions between crest cells (derived from ectoderm) and neighboring tissues (such as endoderm and surface ectoderm), which induce specific bone and cartilage patterns in the face. Recent chick studies showed that head endoderm, which contributes to the lining of the pharynx and gills, can pattern the facial skeleton. But the question remained, by what mechanism does endodermal signaling induce specific patterns of cartilage and bone?

In this issue of *PLoS Biology*, Justin Crump, Mary Swartz, and Charles Kimmel study the patterning of a jaw-support cartilage called the hyosymplectic in the larval zebrafish and find a "hierarchy of tissue interactions" at work. In zebrafish mutated for a gene called *integrinα5*, the authors report, a specific region of the hyosymplectic cartilage fails to develop. The loss of this cartilage region correlates with the loss of the first endodermal pouch. Pouches are outpocketings of the head endoderm that fuse with the skin to form the gill slits later in development. By labeling individual crest cells with fluorescent dye and making time-lapse recordings of these cells in transgenic fish,

Crump et al. show that the hyosymplectic cartilage regions lost in the *integrinα5* mutant are normally derived from crest cells directly adjacent to the first pouch.

Integrins are transmembrane receptors that promote cell adhesion and signaling. Although integrins function in crest cell migration, Crump et al. show that the Integrinα5 receptor is required in endoderm for hyosymplectic cartilage development and appears to promote development of the first pouch. The first pouch in turn acts as a template, by promoting both the survival and local clustering of crest cells, to pattern a specific region of the hyosymplectic cartilage.



DOI: 10.1371/journal.pbio.0020295.g001

Pharyngeal development in a zebrafish embryo

But the pouch may have more far-reaching effects. Since *integrinα5* mutants also have region-specific defects in cranial muscles and nerves, the first pouch may serve to organize an entire functional unit in a region of the head. As the hyosymplectic element has undergone considerable change during evolution—from a jaw-support element in fish to a tiny, sound-conducting bone called the stapes in mammals—Crump et al. speculate that such a local, interconnected strategy of development would facilitate evolution of the vertebrate head. Changes in endodermal signaling would allow a particular skeletal element to vary in shape or size, in coordination with the muscles and nerves that move the skeletal element and independent of other regions of the head.

It will be interesting to determine, the authors note, whether this hierarchical organization applies to other skeletal elements in the head. But for now, these results will inform efforts to understand the specificity of interrelated defects seen in human craniofacial syndromes such as DiGeorge Syndrome, whose underlying causes lie in the development of the endoderm.

Crump JG, Swartz ME, Kimmel CB (2004) An integrin-dependent role of pouch endoderm in hyoid cartilage development. DOI: 10.1371/journal.pbio.0020244

A Developmental Role for Fatty Acids in Eukaryotes

DOI: 10.1371/journal.pbio.0020293

Health food stores have long hawked fish oil capsules as a cure-all for everything from migraines to heart disease. And though such claims are often weak on scientific evidence, fish oil, it turns out, is no snake oil. A recent review of scientific studies concludes that omega-3 fatty acids can indeed protect against heart disease, and the American Heart Association now recommends fish oil capsules for patients with coronary heart disease.

Fatty acids come in hundreds of varieties, distinguished primarily by their structure, which in turn determines their physiological role. Unlike proteins or genes—which are polymers made up of amino acids and nucleotides, respectively—fatty acids are a large group of compounds containing long chains of carbon and hydrogen atoms with a carboxylate group (acid) attached at the end. It is this asymmetrical chemical configuration that gives fatty acids their unique properties. Fatty acid diversity comes from variations in the length of the carbon chain and in the number of double bonds between carbons. Fatty acids with one or more double bonds are called unsaturated fatty acids.

Fatty acids play an essential role in metabolism, providing the cell with a concentrated source of energy, and form the structural foundation of the cell membrane, where they are most conspicuous and perhaps best understood. Long-chain (unbranched) fatty acids, which run ten to 22 carbons long, are the most common fatty acids in animal cells and the most studied. One much less understood class of fatty acids—the monomethyl branched-chain fatty acids (mmBCFAs)—has been found in organisms from bacteria to humans, but its role remains obscure. In this issue of *PLoS Biology*, Marina Kniazeva et al. explore the origin and function of mmBCFAs in the worm *Caenorhabditis elegans* and find that these relatively obscure fatty acids play a crucial role in growth and development.

mmBCFAs are abundant in diverse genera of bacteria, which use a supply of branched-chain amino acids and enzymes to assemble the fatty acid chains. mmBCFA biosynthesis has been characterized in bacteria, but not in eukaryotes. (Worms, and humans, are eukaryotes; our cells have nuclei.) Here, Kniazeva et al. identified worm genes that are homologous to the gene that codes for an enzyme called elongase in another eukaryote, yeast. Elongases are enzymes that extend the length of fatty acid chains by two carbons. To see what kind of fatty acid molecules the homologous worm genes were synthesizing, the authors used a technique called RNA interference (RNAi) to "silence" the genes' expression in the worms. Surprisingly, two of the eight inhibited genes had a specific effect on branched-chain fatty acid levels: *elo-5* and *elo-6*.

Inhibiting *elo-5* function had deleterious effects on the growth and development of the worms. The progeny of worms treated as embryos with RNAi for *elo-5* stopped growing at the first larval stage, while the progeny of worms treated at later stages developed to adulthood but got progressively sicker and showed reproductive problems. These defects were corrected when the researchers fed the mmBCFAs directly to the worms, indicating that these mmBCFAs are essential for normal larval growth and development.

Given the widespread distribution of mmBCFAs in organisms as diverse as bacteria and humans, it's perhaps not too surprising that they regulate essential physiological functions during animal development. It's still not clear, however, what all the components of the fatty acid manufacturing machinery are or how an organism monitors production levels. And though it's still an open question as to how these ubiquitous molecules function in mammals, the fact that they have been conserved throughout evolution underscores their importance—and suggests they may play a similar role.

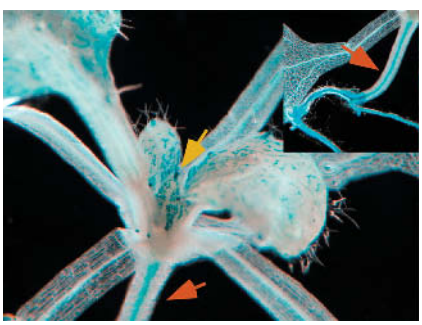
Kniazeva M, Crawford QT, Seiber M, Wang CY, Han M (2004) Monomethyl branched-chain fatty acids play an essential role in *Caenorhabditis elegans* development. DOI: 10.1371/journal.pbio.0020257

Hormones Act in Concert to Direct Plant Growth

DOI: 10.1371/journal.pbio.0020299

Anyone who thinks plants are passive inhabitants of their environment has never seen time-lapse footage of a seedling bursting from its protective shell or a climbing vine coiling around a tree. Such films dramatize a fundamental fact of plant life: survival depends on responding to environmental cues. Shoots grow toward light and against gravity. Stems and roots curl around obstacles that block their paths.

In plants, environmental cues trigger hormonal changes that in turn regulate cells' shapes and proliferation. In this way, subtle changes in the environment affect plant growth. Auxin, the first known plant hormone, spurs growth and shapes growth patterns in nearly every plant tissue throughout a plant's lifecycle. Brassinosteroids—a class of hormones chemically similar to animal steroids like testosterone—are linked to many of the same processes as auxin.



DOI: 10.1371/journal.pbio.0020299.g001

Emerging leaf tips (yellow arrow) and hypocotyl (orange arrows) of an *Arabidopsis* mutant

Early physiological and molecular experiments gave conflicting evidence about whether auxin and brassinosteroids had similar effects. For many years, biologists believed that these hormones acted through independent signal transduction pathways—chains of molecules that relay stimuli and elicit cellular responses, such as gene expression. But in the last few years, microarray studies, which can measure the transcription of thousands of genes simultaneously, showed that auxin and brassinosteroids do regulate expression of several genes in common.

In this issue of *PLoS Biology*, Jennifer Nemhauser et al. assay the entire genome of *Arabidopsis thaliana*, a favorite for plant genetics studies, for effects of auxin and brassinosteroids. The group's microarray analyses show that these hormones affect transcription of about 80 genes in common—including many known players in the hormones' signal transduction pathways. To see how this regulation could occur, the research team looked at the genes turned on by both hormones to find common promoter sequences—regions of the genome that do not code for protein but instead help regulate gene transcription. They used a new computational approach to tease out promoter regions that auxin and brassinosteroid pathways both act upon, showing how these hormones have overlapping effects on gene transcription.

The group also compared the effects of auxin and brassinosteroids on seedlings' stem growth and gene expression in a variety of mutant *Arabidopsis* lines. They showed that auxin and brassinosteroids greatly enhance each other's effects on stem growth, demonstrating that the interaction of these hormones is important for normal plant development. Mutants with a disabled auxin pathway don't respond normally to brassinosteroids, and vice versa. Also, mutants with abnormally high levels of auxin have a reduced number of genes that respond to brassinosteroids. Thus, these hormones act through overlapping, interdependent pathways—but they don't regulate each other directly. Instead, the researchers suggest, the pathways likely converge on the promoters of a few key genes.

It's still an open question why plants use these hormones with such redundant effects. Nemhauser speculates that—as is known to be the case in animals—by having dual, interdependent pathways, plants can finely tune how these ubiquitous hormones act in different cells and tissues to shape patterns of growth. By showing clearly that auxin and brassinosteroids act together and how they affect many of the same genes, Nemhauser and colleagues have set the stage for more detailed studies of how these hormones act in specific parts of plants to shape growth.

Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. DOI: 10.1371/journal.pbio.0020258

Policing Relative Conflicts of Interest in Social Insects

DOI: 10.1371/journal.pbio.0020324

The order and harmony that appears to bless the lives of many social insects, from ants to bees, has long fascinated naturalists. That individual workers seem to routinely sacrifice their own (reproductive) interests for the good of the colony has also piqued the interest of philosophers and kings, for obvious reasons. But scratch the surface and that blissful harmony reveals a complex feat of social engineering that is both exquisitely organized and potentially ruthless.

One of the altruistic behaviors that social insects are famous for is that one or a few queens perform most or all of the reproduction in a colony, while workers are, for the most part, non-reproductive. The evolution of this social structure partly stems from the unusual sex determination system of social insects, in which unfertilized eggs (of either workers or queens) develop into males and fertilized eggs (produced only by queens) develop into female queens and workers. This creates unusual relationships between family members that affect how W. D. Hamilton's theory of "kin selection" operates in these species. Kin selection, as elegantly summarized by "Hamilton's Rule," predicts that the altruistic behavior of workers—that is, investing in the reproduction of others in the colony rather than in their own reproduction—can evolve if the indirect reproductive payoff to workers (i.e., via reproduction by relatives) is higher than the cost of the missed opportunity for direct reproduction. Kin selection revolves around relatedness because relatedness determines the magnitude of indirect reproductive payoffs. However, based on a survey of 50 species of ants, wasps, and bees, Rob Hammond and Laurent Keller now demonstrate that the behavior in the colony cannot be accounted for simply based on relatedness patterns, but that it is necessary to consider how colony efficiency influences behavior.

In some social insect colonies, workers do lay eggs, in a sense "cheating" on the other workers who are investing in the queen's reproduction rather than in their own. Such action can be severely penalized by other workers, who aggressively police the colony for the

10.1371/journal.pbio.0020324.g001

Conflict between ants (Photo: Christian König, www.konig-photo.com)

illicit offspring—or behavior—of their guilty colleagues. In honeybees, where this behavior was first shown, workers remove worker-laid eggs within hours by eating them, and, in some ants, more draconian methods lead to the mutilation of the culprit caught in the act of laying.

Why workers police worker reproduction in some colonies and not in others can also be influenced by relatedness. If a queen is monogamous and mates only once, then each worker will actually be more related to her nephew (produced by a sister worker) than to her brother (produced by the queen); in this case, workers should tolerate other workers' male offspring. But if the queen mates more than twice (as in honeybees) or if there are multiple queens heading a colony, then the relationship between workers becomes diluted (they do not all have the same father), and workers are more closely related to brothers than to nephews. In this case, workers should clamp down hard on any worker breeding and raise only the queen's sons (in addition to her daughters).

But workers policing the reproduction of their fellow workers could also be advantageous if the energy invested by workers into laying eggs—which would otherwise be used in foraging and legitimate brood rearing—detracts from the overall efficiency and growth of the colony. Although there is some evidence for this "efficiency hypothesis," it is widely accepted that the driving force behind policing is primarily explained by patterns of relatedness. By doing a detailed comparative phylogenetic analysis of different species, Hammond and Keller put the "relatedness hypothesis" to the test and—contrary to expectations—found evidence that this genetic incentive for workers to police the reproduction of other workers cannot account for its widespread prevalence among social insects.

One prediction from the relatedness hypothesis is that the extent to which workers produce male offspring is determined by the relatedness of the workers. By contrast, the efficiency hypothesis predicts no such relationship. In line with this, Hammond and Keller's survey reveals that no matter how related workers are to each other, most males across this broad range of species are produced by queens. In other words, worker-policing does not depend on relatedness, so other factors—such as colony efficiency—must act as an important constraint on worker reproduction. This, Hammond and Keller emphasize, does not amount to showing that kin selection is unimportant—but it does mean that the harmony and regulation of reproduction in social insects is much more complex than expected from simple theoretical expectations based solely on relatedness.

Hammond RL, Keller L (2004) Conflict over male parentage in social insects. DOI: 10.1371/journal.pbio.0020248

The Case of the Noisy Neurons

DOI: 10.1371/journal.pbio.0020314

People are unpredictable. One night you may crave Italian food, but another only Thai will do. One day you might finish a crossword puzzle in record time, and the next not a single clue prompts an answer. Such behavioral variation has been found in laboratory studies, too: a person's ability to find a faint image on a screen varies widely from one viewing to the next. Similarly, when an animal repeatedly receives the same stimulus—for example, a faint image—a neuron in a region of the animal's visual brain might be very active upon one presentation and relatively quiet the next.

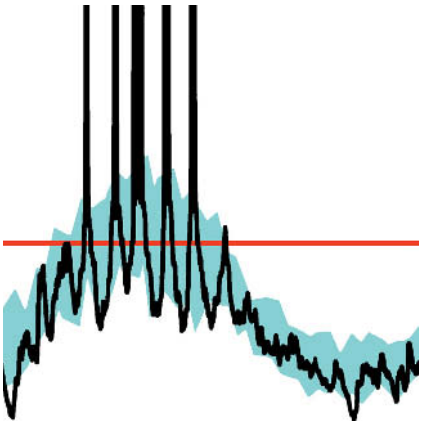
Across the cerebral cortex—the brain region that integrates the senses and controls voluntary movement—neurons are notorious for their unpredictable behavior. The neurons themselves don't create this noise; when directly stimulated with an electrode multiple times, neurons will give the same response every time. Most neurons, however, receive signals from a host of other neurons. These various signals combine to form a seemingly noisy electrical input, which shows up as fluctuations in the recipient neuron's membrane potential—a difference in electrical charge between the inside and outside of the cell's membrane. Neuron function is intimately tied to the membrane potential, which is usually maintained within a narrow range, called the resting potential. But incoming signals can push the resting potential higher or lower. If the membrane potential rises above a certain threshold, the neuron fires, sending an electrical signal down its length. In this way, the brain relays and processes information.

Since the 1960s, neuroscientists trying to account for the cortex's variable responses have pointed to noisy inputs from other parts of the brain as the prime suspect. In this issue of *PLoS Biology*, Matteo Carandini addresses this long-standing mystery of neuron variability and comes up with a different answer. Carandini simultaneously measured the membrane potentials and firing patterns of individual neurons in the cat visual cortex. He found, surprisingly, that the membrane potentials varied much less than the firing patterns, ruling out noisy inputs as the cause of neurons' noisy

outputs. Instead, the neurons amplified noise in the signals they received.

Carandini then used a simple model of neuron behavior to explain why this would occur. He started with a tried-and-true approximation of neuron behavior, called the rectification model: a neuron doesn't fire until its membrane potential rises above a threshold, but once it crosses this threshold, its firing rate is correlated with the strength of incoming signals. Then he added the assumption that the neurons receive signals with some randomness. Given these minimal assumptions, Carandini showed that neurons fed a noisy signal will tend to amplify the noise in the signal. Importantly, his model reproduced a well-known phenomenon: as cortical neurons' average firing rate goes up, their firing rate also becomes more variable—that is, they get noisier.

Carandini's model also predicted something new: as the firing rate continues to increase, the firing rate should become more consistent and less noisy—which he calls saturation of variability. Carandini's measurements in cats showed neurons actually behave this way, a key validation of his model.



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Noise and threshold make neurons unpredictable

It's not clear whether this amplification of variability is something that helps or hampers the brain. Despite being a nuisance to neuroscientists, such fluctuations could be crucial to how the brain functions, Carandini speculates. Without some variability in their cortex, animals would act like cameras or other simple machines that respond the same way each time to a stimulus. It's advantageous for behavior, and hence brains, to be adaptable. But amplifying noise in a signal seems to run counter to relaying and processing the information in the signal. Carandini suggests that what appears as noise in the experiments are signals from other parts of the cortex—that is, noise is in the eye of the beholder. Now that the source of the variability is clear, neuroscientists can study whether it serves a function in the brain.

Matteo Carandini (2004) Amplification of trial-to-trial response variability by neurons in visual cortex. DOI: 10.1371/journal.pbio.0020264