One reason to sequence the genomes of non-human organisms is to better understand our similarities and differences. And, at first sight, it is hard to imagine a eukaryote more different from humans than *Tetrahymena thermophila*. A relative of *Paramaecium*, this single-celled creature has a strong but flexible exterior covered with rows of cilia; but it is inside where things seem to get really alien. Each cell contains not one but two nuclei: a micronucleus, which contains only five chromosomes, and a macronucleus, which has more than 200.

Biologists have long known that the micronucleus contains the DNA reserved for reproduction, and that the macronucleus arises from the micronucleus and controls the cell’s other functions. During macronucleus formation (which happens each time the cells mate), each of the five chromosomes splinters into multiple fragments, which in turn replicate to form many copies of the resulting smaller chromosomes. In a new study, Jonathan Eisen and a team of over 50 scientists report the full sequence of the macronuclear genome.

The authors began by isolating DNA from purified macronuclei (no mean feat in itself), and performed a “shotgun” sequence, splitting the DNA into millions of fragments, sequencing each of these, and then reconstructing the whole by using computers to match overlaps. They estimate that they have captured more than 95% of the genome, and conclude it is 105 million base pairs in length. The exact number of chromosomes is still at issue, though the authors present evidence that it lies between 185 and 287, and, based on the number of telomeres, is probably about 225.

* T. thermophila macronuclear chromosomes, unlike those in the micronucleus and other species, are highly unusual because they appear to lack centromeres, the regions that link chromosomal replicants and then guide their separation during mitosis and meiosis. This makes some sense, since the macronucleus undergoes neither process. Furthermore, they contain much less repetitive DNA than most other eukaryotes—about 2% of the total DNA, versus over 50% in humans—partly because most repetitive DNA is jettisoned during the formation of the macronucleus, when about 15% of micronuclear genomic DNA is excised. The authors provide evidence that excision targets not only repeated elements per se but also foreign DNA (such as “selfish” mobile DNA transposons) in particular, indicating the importance of this process in maintaining the integrity of the expressed genome from such outside invasions.

Sequencing the genome also allowed the authors to address a nagging evolutionary question, namely the timing of plastid acquisition in the alveolates, a group of three related phyla: the ciliates (including *Tetrahymena*), the apicomplexans (parasites that cause malaria, among other diseases), and the dinoflagellates (ocean-dwelling photosynthetic protozoans). Plastids, such as the chloroplast, are organelles descended from what were once free-living cyanobacteria; typically, many of the genes of such an endosymbiont are shifted into the host nucleus, as they have been in the apicomplexans and dinoflagellates. *T. thermophila* has no plastids, but it has been suggested that its ancestors did. The authors discovered no remnants of plastid genes within *T. thermophila*, strongly suggesting that plastid acquisition occurred after the other two groups split off from the ciliates.

All told, the genome contains over 27,000 protein-coding genes, more than naively expected for a single-celled species and comparable to the number in humans. Certain gene families appear to have expanded significantly in *T. thermophila*, indicating the likely importance of the processes carried out by the proteins each family encodes. An example is the presence of over 300 genes for voltage-gated ion channels, which control membrane transport, a key function of this free-living, single-celled creature. Previous analysis of gene structure showed that *T. thermophila* uses only one stop codon (UGA) during protein synthesis, compared to the three that are standard in most eukaryotes; the unused ones instead encode glutamine. As in many other organisms, UGA itself is also used in some genes to encode the amino acid selenocysteine, making *T. thermophila* the only known organism to translate all 64 codons.

The authors also wish to sequence the micronuclear genome, which should provide insights into *T. thermophila* biology that is unavailable from the macronucleus alone. A key component of the project is that all of the data have been made publicly available without restrictions throughout the project, allowing the scientific community to freely analyze the genome of this organism even prior to this publication.

Eisen JA, Coyne RS, Wu M, Wu D, Thiagarajan M, et al. (2006) Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. DOI: 10.1371/journal.pbio.0040286
The tiny nematode Caenorhabditis elegans spends most of its life in the soil, searching for an abundance of food and just the right amount of oxygen. But what happens when optimal oxygen and food supplies can’t be found in the same place? More generally, how does the organization of an animal’s neural networks help it produce the right behaviors in competing contexts?

With only 302 neurons, and powerful genetic tools available to the researchers who study it, C. elegans is a valuable subject for exploring the neural control of behavior. Previous work has identified just three kinds of neurons as important for sensing and responding to oxygen. These neurons express a family of genes that appear to encode enzymes called soluble guanylate cyclases (sGCs). C. elegans sGCs bind oxygen and initiate signaling cascades within the neurons. Animals lacking certain members of this gene family no longer respond normally to oxygen. But, since other neurons also express sGCs, these neurons could play a role in oxygen sensing as well.

C. elegans’ response to high ambient oxygen (above 14%) in the presence of food depends on the activity of a neuropeptide receptor called NPR-1. Naturally occurring npr-1(215F) nematode strains and laboratory-induced npr-1(lf) strains avoid high oxygen whether or not food is present and aggregate in the presence of food. Another naturally occurring strain, npr-1(215V), avoids high oxygen only when food is absent. How does npr-1(215V) integrate the information about the two stimuli? To learn the answer, Andy Chang, Cornelia Bargmann, and colleagues systematically assessed the possible role of a number of neurons and genes using mutation and selective gene replacement. Their experiments involved first removing the function of a particular gene (for example, an sGC), then assessing the change in response to oxygen (by looking for changes in the typical distribution of animals along an oxygen gradient), and then finally replacing that gene in only one kind of neuron to see if normal function returns.

Their results revealed some surprises. Previous studies showed that the neurons URX, AQR, and PQR suppress npr-1(215V)’s locomotor response to oxygen. In this study, the researchers found another set of neurons—SDQ, ALN, and PLN—expressing sGCs that were able to process information about ambient oxygen levels. They also found that the ion channels OSM-9 and OCR-2 in yet another set of neurons (ADF and ASH) promote high-oxygen avoidance. The researchers concluded that these neurons interact with sGC neurons to produce high-oxygen avoidance and modulation of this response by food.

Another aggregating strain of C. elegans, daf-7, gave the researchers yet another angle to explore. In crowded, low-food conditions, the developmental gene daf-7 shows low activity and the nematode enters an alternative larva stage called a dauer. The researchers found that daf-7 mutants avoided high oxygen with or without food, suggesting that daf-7, like npr-1(215V), is involved in suppressing high-oxygen avoidance in the presence of food. Further studies suggested that food might be exerting its influence in part by altering daf-7 expression in ASI neurons. The researchers also found that daf-7 mutants expressed higher levels of a gene involved in serotonin synthesis in ADF neurons, suggesting that ADF may represent a convergence point for networks that promote response to high oxygen and those that suppress it.

The researchers concluded that at least four sets of sensory neurons (some or all of URX, AQR, and PQR; some or all of SDQ, ALN, and PLN; ADF; and ASH) in C. elegans promote high-oxygen avoidance, and that these neurons can be suppressed in some cases by other neurons that provide information about food availability. The result is an integrated system that allows this simple organism to respond to its complex environment in an equally complex manner. Electrophysiological examination of other “simple” systems, like motor circuits in the leech and the lobster, has demonstrated comparable complexity in well-defined neural networks, with context-dependent neuronal participation in a particular behavior. The principles uncovered in these systems are likely to be applicable to even more complex brains, whose neuronal circuits are not amenable to comparable dissection.

Chang AJ, Chronis N, Karow DS, Marletta MA, Bargmann CI (2006) A distributed chemosensory circuit for oxygen preference in C. elegans. DOI: 10.1371/journal.pbio.0040307

Bacterial Fimbriae Designed to Stay with the Flow

Liza Gross

DOI: 10.1371/journal.pbio.0040314

The human digestive system houses a diverse colony of beneficial bacteria, but one species—E. coli—can wreak havoc when it colonizes mucous membranes that normally exist unmolested (for example, in the urinary tract). To latch on to cells and establish infection, E. coli uses fimbriae—long, hairlike organelles that project from the bacterium’s surface. Fimbriae consist of interlinking subunits of a single protein called pilin that forms a rigid, coiled helix-shaped rod. Sticky proteins called adhesins cap the tip of the rod and bind to carbohydrate receptors on their host, thus securing bacteria on the host cells as extracellular fluids swirl around them.

A previous study led by Evgeni Sokurenko and Viola Vogel...
investigated the most common type of *E. coli* fimbriae. The sticky protein at the tip of these fimbriae is called FimH and binds to a carbohydrate called mannose. They showed that powerful drag forces created by the extracellular fluids don’t carry the bound bacteria away, as one might expect, but instead strengthen their adhesion to their host. The researchers attributed this increased binding to a biphasic “catch bond” mechanism whereby increased drag forces cause the FimH at the tip of the fimbria to switch from a form that binds mannose weakly to a form that binds strongly. Because of this, the bacteria bind best at an optimal force that is high enough to switch FimH to strong binding but not so high that it breaks the strong FimH–mannose bond.

And now, in a new study, the same group of researchers (including first author Manu Forero) set out to determine whether the coiled rod structure of fimbriae affects how the sticky FimH at the tip binds. It had been assumed that fimbrial rods play a largely static structural role, either by extending the tip adhesins’ reach or by resisting electrostatic repulsive forces between bacteria and cell surfaces. But Forero et al. show that the rods function more dynamically, using their mechanical properties to help stabilize the FimH–mannose bond against a turbulent background.

Fimbriae-mediated adhesion was investigated with an atomic force microscope, which uses a cantilever to apply (and measure) forces between its tip and the sample under investigation. Forero et al. outfitted the cantilever tip with mannose, and then used this to touch a fimbriated *E. coli* cell that was affixed to a glass surface. After mannose bound to the fimbrial FimH, the cantilever retreated from the bacterium at a constant velocity. The researchers determined that, instead of the FimH–mannose bond breaking, the fimbriae stretched out far beyond their original length.

One reason that fimbriae extend could be that the individual pilin subunits of the fimbrial rod are uncoiling. The researchers tested this hypothesis by applying a constant force between the cantilever and fimbria—under which fimbrial length changes slowly. They observed that stepwise jumps in distance corresponded to the expected length of individual subunits unwinding from the coiled shaft one at a time. Thus, the researchers concluded, fimbrial elongation proceeds as subunits uncoil one after another. This was also supported by a mathematical model developed by the researchers to quantify the biophysical forces governing the dynamics of fimbrial uncoiling.

Forero et al. also detected that, after uncoiling at increasing force, the stretched fimbriae re-coil if the pulling force drops. Importantly, while fimbrial uncoiling under high force decreases the tension within the rod, re-coiling under low force increases the tension. Thus, the tensile force within the rod stays within some intermediate level when fimbrial length is stable.

The researchers found that the intermediate force range corresponds to the force level where the FimH–mannose bonds last longest. Lower, uncoiling forces are too weak to switch bonds to a long-lived state before breaking, and higher, uncoiling forces exceed the catch-bond threshold, shortening the life of the bond. Because *E. coli* living in the gut or other mucosal surfaces experience constantly changing flow rates and forces, these adjustments should enhance fimbrial attachment under a diverse range of fluid conditions. The correspondence of forces suggests that the mechanical properties of the fimbrial rod and the FimH–mannose complex co-evolved to optimize adhesive stability in fluids.

Forero M, Yakovenko O, Sokurenko EV, Thomas WE, Vogel V (2006) Uncoiling mechanics of *Escherichia coli* type I fimbriae are optimized for catch bonds. DOI: 10.1371/journal.pbio.0040298
Anatomy of a Fever

Liza Gross | DOI: 10.1371/journal.pbio.0040305

Many parents experience fear and anxiety when their child comes down with a fever, unaware that fever is an ancient, often beneficial, response to infection. The fever response is conserved across all mammals and many vertebrate classes.

(Even reptiles and other cold-blooded animals fare better against infection when they develop fever by soaking up the sun’s heat.) Among other potential adaptive benefits, a higher temperature can inhibit the growth of bacterial strains that lack sophisticated mechanisms for coping with heat shock.

Fever, which is mediated by a lipid called prostaglandin E₂ (PGE₂), can pass through multiple temperature phases. While it’s well established that PGE₂ originating in brain cells causes the second and later phases, the initial phase of fever has proven difficult to characterize. Of particular interest is whether fever onset is triggered by PGE₂ that originates inside or outside the brain—a question that has dogged researchers for nearly three decades. Now, Alexandre Steiner, Andrej Romanovsky, and colleagues provide evidence that PGE₂ synthesis doesn’t begin in the brain as previously thought, but in the lungs and liver. They also describe the molecular mechanisms that produce PGE₂ in these organs.

Many of the mechanisms of fever have been established by exposing rodents to bacterial endotoxins called...
lipopolysaccharides (LPS). The first phase of LPS-induced fever starts within 30 minutes after exposure to the pyrogen. During the second and subsequent phases—between 90 minutes and 12 hours after LPS administration—brain cells increase production (called upregulation) of enzymes involved in PGE$_2$ synthesis. Thus, fever starts about an hour before the PGE$_2$- synthesizing enzymes—cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1)—are upregulated in the brain, suggesting that fever must be triggered by PGE$_2$, produced peripherally, outside the brain.

To test this hypothesis, Steiner et al. gave rats an intravenous (IV) solution of PGE$_2$ bound to albumin, the primary transporter of PGE$_2$ in the blood. Controls received an IV albumin solution. After confirming that the PGE$_2$ infusion induced fever, the researchers collected venous and arterial blood from LPS-treated rats. (PGE$_2$, synthesized in tissues amasses in venous blood; arterial blood delivers PGE$_2$ to the brain.) At the onset of fever, PGE$_2$ was significantly elevated both in the venous and arterial blood.

To investigate the origin of fever-inducing PGE$_2$, Steiner et al. used an antibody-based technique. (Antibodies are too large to cross the blood–brain barrier.) Rats were pretreated with IV anti-PGE$_2$ antibody serum (controls received normal serum) and then injected with IV LPS. The first phase of LPS fever was significantly attenuated by the antibody (but not the serum), which was found in the blood but not in the brain. These results show that LPS-induced fever is triggered by circulating PGE$_2$.

A previous study from the Romanovsky laboratory reported that onset of fever is accompanied by significantly increased transcription of COX-2 and mPGES-1 in the lung and liver, and a moderate increase of the COX-2 transcript in the hypothalamus (the body’s “fever center”). In this study, they examined the protein distribution of these enzymes in all three tissues. After LPS exposure, the content of neither protein was increased in the hypothalamus. But the lung and liver did show increased COX-2 expression—primarily in macrophages, which play a role in the inflammatory response—along with activation of an enzyme (cytosolic phospholipase A2) involved in the early steps of PGE$_2$ synthesis. These organs, but not the hypothalamus, also showed signs of inflammatory signaling. Interestingly, the researchers did not find increased levels of mPGES-1, which facilitates the final step of PGE$_2$ synthesis, suggesting that the cell’s normal supply of this enzyme manages the task.

Altogether, these results provide a cellular and molecular portrait of the first phase of fever and show that it depends, at least in part, on PGE$_2$ that originates in peripheral tissues.

Steiner AA, Ivanov AI, Serrats J, Hosokawa H, Phayre AN, et al. (2006) Cellular and molecular bases of the initiation of fever. DOI: 10.1371/journal.pbio.0040284
Green fluorescent protein traces different types of lipofected cells in the neural retina of a Xenopus tadpole, some of which (ganglion cells) generate fibers of the optic nerve.

Working with Xenopus frogs, a classic developmental biology model, they found that each of the homeobox genes was expressed in sequence, in different cells. By mid-stage retinal development (stage 34), the messenger RNA (mRNA) transcripts of all three genes were expressed, but only Xotx5 proteins were detected. Xvsx1 and Xotx2 were detected at stages 37 and 38-39, respectively. By stage 42, Xotx2 and Xvsx1 proteins were observed in bipolar cells, while Xotx5b was detected only in photoreceptors. These results indicated that the genes had been regulated after transcription and were expressed as proteins after cells exited the cell cycle.

What controlled the genes’ translation into protein? To find out, the researchers linked a specific sequence of each homeobox gene—called the three prime untranslated region (3’ UTR)—with the gene encoding green fluorescent protein (GFP). These GFP sensors indicated how the 3’ UTR affects expression of the gene. They delivered the DNA of sensors into embryos at an early stage of retinal development (stage 17-18), using a technique called lipofection. GFP proteins were detected only in photoreceptors (the Xotx5b sensor) and bipolar cells (Xvsx1 and Xotx2 sensors). Thus, the 3’ UTRs of these genes had blocked GFP translation into protein in all but late-developing retinal cells. The 3’ UTRs were able to do this because they contain sequences (called cis-regulatory sequences) that can interact with microRNAs—a class of gene-repressing RNAs that bind to complementary sequences of RNA and mediate mRNA destruction. (Future experiments must confirm whether these sequences do in fact interact.) The GFP sensors were detected at the same stages as their corresponding homeobox proteins had been in the previous experiments. This timing, it turned out, coincided with the birthdates of the photoreceptors and bipolar cells.

The correlation between the timing of protein expression and the photoreceptor and bipolar cell birthdates prompted the researchers to examine the effect of cell cycle progression on protein translation. By blocking cell cycle progression with drugs that inhibit DNA replication, they found that Xotx5b, Xvsx1, and Xotx2 require progressively longer cell cycles for efficient translation. And the attenuated production of Xotx5b and Xvsx1 proteins after cell cycle inhibition, they found, reduced the number of photoreceptor and bipolar cells—an effect that was reversed when the proteins were overexpressed, supporting the connection between protein expression and cell identity.

Altogether, these results indicate that a post-transcriptional mechanism regulates when these proteins are expressed and in which cells. This mechanism operates in synch with a cellular clock that measures cell cycle length to generate the later developing photoreceptors and bipolar cells. The next step will be to determine how these findings apply to other genes controlling retinal cell fate, and then to identify the molecular mechanisms driving translational inhibition.

Decembrini S, Andreazzoli M, Vignali R, Barsacchi G, Cremisi F (2006) Timing the generation of distinct retinal cells by homeobox proteins. DOI: 10.1371/journal.pbio.0040272

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Your Synapse or Mine? Promiscuous Protein Exchanges between Neighboring Synapses

Françoise Chanut | DOI: 10.1371/journal.pbio.0040297

Synapses, the connections that link neurons into circuits, can be plastic or stable in the mammalian brain. Right after birth, synapses form and dissolve among nascent neurons at breakneck speed as the animal adapts to its new surroundings. But, over time, while some plasticity remains and allows for learning, most synapses stabilize and some may last a lifetime. How synapses are maintained over such long periods is somewhat of a mystery, especially in light of the fact that structural proteins are constantly moving in and out of synapses. In theory, the active turnover of synaptic components might simply reflect the balance between protein synthesis and degradation. But, in a recent study, Shlomo Tsuriel, Ran Geva, Noam Ziv, and their colleagues find that two prominent synaptic proteins, Synapsin I and ProSAP2, turn over primarily through rapid exchanges between neighboring synapses, rather than via synthesis and degradation. These observations add an interesting twist to the already complex picture of synapse biology.

Synapses are specialized devices that serve to transfer electrical impulses between neurons. They form at discrete contact points between the neuron’s main branch (the axon) and the complex arborizations (dendrites) that sprout from its target neuron’s cell body. A number of specialized structures and molecules accumulate...
In the game of survival, anything goes—even the selective extermination of males. Male killing is the preferred strategy for a diverse group of bacteria that infect insects and other arthropods. Aside from its tabloid appeal, male killing offers biologists a platform for investigating genetic conflict—evolutionary battles between competing elements within the same genome. Male-killing bacteria are passed from mother to offspring, but only males die from infection, suggesting that males harbor genetic elements that allow them to succumb to infection. In keeping with evolutionary theory, these selfish genetic elements, which spread at the expense of the organism, should engender countering elements that promote male survival. Yet scant evidence has linked the evolution of host suppressors to selfish elements that mediate male killing.

But now, Emily Hornett, Gregory Hurst, and colleagues report the first case of total suppression of male killing in a butterfly, *Hypolimnas bolina*, infected with the *w*011 strain of the male-killing bacterium *Wolbachia*. They attribute survival to genetic elements expressed in the male embryo, an effect called zygotic suppression. Because this mechanism of suppression can inactivate male killers—which lie dormant until presented with a novel, vulnerable host—it’s possible
that insects that don’t succumb to male killing today may have in fact evolved the means to counteract lethal infection. 

*H. bolina* is found throughout the Indo-Pacific. Because *w*Bol1 infection kills males in Polynesia but not in Southeast Asia, breeding individuals from each region could reveal genetic elements in the different populations that favor life over death. And because infected females transmit infection directly to offspring, breeding could also introduce *w*Bol1 genes (and infection) onto the butterfly genetic background (a technique called introgression).

The breeding experiments tested two questions: would male-killing *w*Bol1 taken from Moorea in Polynesia lose that ability against Southeast Asian males with a Thai or Philippine genetic background, and would benign *w*Bol1 from Thailand or the Philippines turn lethal against males with a Moorean genetic background?

To find out, Hornett et al. mated infected Moorean females with Thai and Philippine males, and mated infected Thai and Philippine females with Moorean males. As a control, *w*Bol1-infected females from both regions were also crossed with males from their native populations. Crossing the Moorean and Southeast Asian populations suppressed the male-killing effects of *w*Bol1 from Moorea in just a single generation—in stark contrast to the control crosses (Moorean females mated with Moorean males), which yielded no males at all.

But when Moorean *w*Bol1 infection was reintroduced to its native host background—by backcrossing first-generation hybrid Moorean/Southeast Asian females with wild Moorean males—it became male-lethal again. Egg hatch rates decreased dramatically and just a fraction of males survived. In contrast, continued introduction of Moorean *w*Bol1 infection onto the Southeast Asian male genetic background produced high hatch rates and a normal sex ratio.

Infected Thai and Philippine females were serially mated with Moorean males, thus progressively increasing the proportion of Moorean genetic material. By the second generation, some male killing occurred, and by the third generation, males were killed in five out of 15 crosses. By the fifth generation, no males survived.

From these results, the researchers concluded that suppression occurs in the embryo, because male offspring of Moorean females crossed with the Southeast Asian males survived even though the mother’s genetic profile allows killing. The fact that first-generation hybrids survived at nearly the same ratio as seen in wild Southeast Asian males, they explain, suggests the effect is dominant (requires just one copy of the gene) and is at high frequency in the population. A dominant effect also explains why male killing didn’t occur in first-generation crosses between Southeast Asian females and Moorean males—the suppressor elements had not been segregated out of the population yet.

Through simulations, the researchers show that the suppressor could spread through the population in just 100 generations, suggesting that male killing could disappear relatively quickly after a suppressor mutation occurs. Thus, genetic conflict between killing abettors and suppressors may be far more widespread than once thought, but has simply eluded detection. Given the diversity of species afflicted by male-killing bacteria, researchers will have plenty of options for testing this possibility.

Hornett EA, Charlat S, Duplouy AMR, Davies N, Roderick GK, et al. (2006) Evolution of male-killer suppression in a natural population. DOI: 10.1371/journal.pbio.0040283

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**In Drosophila Hair Development, Shavenbaby Is at the Beginning of the End**

Richard Robinson | DOI: 10.1371/journal.pbio.0040310

In the past two decades, the grand strategy of animal development has become clear: initial sets of transcription factors turn on some genes and turn off others, creating new sets in turn, at each step defining the fate of cells ever more precisely as embryonic development proceeds. At the end of this process, a fully differentiated cell with a characteristic shape emerges, but the signals that bring about these final steps have often remained elusive. A new study by Hélène Chanut-Delalande, Serge Plaza, and colleagues deciphers these signals for epidermal hair formation in *Drosophila*, illuminating the link between the cascade of transcription factors and the production of a specific cell shape in this model animal.

The embryo of the fruit fly is divided into parallel segments. The epidermal surface of each segment may be smooth, or studded with projections known as trichomes. Called denticles on the ventral surface and hairs on the dorsal surface, trichomes arise from extensions of the cytoplasm of individual cells, and are filled with the cytoskeletal protein actin. Previous work has shown the importance of several transcription factors in trichome formation, converging on Shavenbaby (Svb), the most “downstream” regulator of trichomes yet identified (svb mutants do not form trichomes, giving the embryos a “shaven” look).

To find the downstream targets of Shavenbaby, the authors examined gene expression patterns in *Drosophila* epidermis, looking for genes whose expression correlated in space and time with that of trichome formation. A gene called *miniature* matched the pattern closely. They showed that *svb* mutation abolished *miniature* expression, as did a repressor of *svb* activity. When they expressed *svb* in cells

where it is normally silent, *miniature* was also expressed. And in a species with restricted *svb* expression, the pattern...
of restriction was matched by restricted miniatura expression as well. The sine qua non of a transcription factor is its ability to directly interact with its target DNA. The authors showed that the Svb protein was indeed able to bind with a small region of the miniatura gene, influencing its transcription.

But is miniatura the only gene Shavenbaby controls in trichome formation? When deleted, denticles still form, but are misshapen, and when expressed where it is normally silent, it was not sufficient to form denticles by itself. From this, the authors deduced that Shavenbaby must have other targets besides miniatura that control denticle formation. Beginning with database searches and continuing with molecular analysis, they found a small handful of genes specifically activated by Shavenbaby and involved in formation of denticles, each of which helps control dynamics of actin reorganization in the epidermis.

No single gene mutation abolished denticle formation, but if all were mutated, denticles (or dorsal hairs) were either tiny and misshapen, or altogether absent, suggesting that collectively, the identified genes were in charge of trichome formation. miniatura, their experiments showed, does not control actin dynamics, but acts at the epidermal cell membrane to regulate the interaction of cytoskeletal elements with the overlying hard cuticle layer. Thus, Shavenbaby controls both actin-related genes and at least one other gene critical for formation of the final shape of the epidermal cell. Finally, the authors showed that Shavenbaby also helps control pigmentation of denticles cells, through regulation of a gene in the pigment synthesis pathway.

From these results, the authors propose that Shavenbaby regulates a “morphological module” that directly influences epidermal form. They note that Shavenbaby’s role in forming both denticles and dorsal hairs, which have different shapes, indicates the flexibility of the module, suggesting the module may be used elsewhere as well, and that the fine tuning that produces one or another type of trichome is likely done by elements both up- and downstream from Shavenbaby. It is likely that other genes, yet to be identified, are also regulated by Shavenbaby, and that some or most of these may be involved in actin remodeling or other aspects of epidermal shape determination. And now, researchers have a platform for investigating these questions.

Sharing Responsibility for Clathrin Coat Assembly

Liza Gross | DOI: 10.1371/journal.pbio.0040301

Membranes protect cells from extracellular insults, but in so doing also block entry to nutrients and other essential molecules. One way cells circumvent this problem is by selectively binding such molecules to receptors on the membrane, then pulling the whole lot into the cell and packaging them into vesicles. Clathrin molecules—three-pronged pinwheel-shaped proteins—form an elaborate lattice coat around the vesicles, which ultimately bud off from the membrane and transport their cargo to their cellular destination.

This highly complex process, called clathrin-mediated endocytosis, requires a constellation of accessory proteins that interact with key protein hubs. Vesicle formation has traditionally been described as a linear process with the core proteins being clathrin and adaptor protein (AP) complexes. In a previous paper, Harvey McMahon and colleagues suggested that the process can be viewed as a network of protein interactions with clathrin and APs forming the two main hubs of the network. In a new study, Eva Schmid, Marijn Ford, McMahon, and colleagues use an impressive array of tools—biophysical, biochemical, structural, and cell biological—to shed light on the network dynamics of this “endocytic interactome.” APs orchestrate the process of cargo recruitment and assembly of the nascent vesicle and are the first hub of the endocytic network. They found that clathrin takes over from adaptors as a hub as clathrin assemblies into a coat. This shift requires collaboration between the hubs, which operate within a dynamic network that performs multiple tasks simultaneously.

Of four AP complexes involved in cellular transport, AP2 figures mostly in plasma membrane endocytosis. The AP2 structure has long been likened to Mickey Mouse, with the four-subunit core representing Mickey’s body and the two flanking appendages forming his ears, but mounting evidence suggests the British children’s book character Mr. Tickle—a circular blob with gangly, elastic arms and little hands—may be a more apt comparison. Mr. Tickle’s body is the core, his arms are the two flexible hinge domains, and his hands are the two appendages, β-appendage and α-appendage. Whichever character you prefer, the core anchors the complex to the membrane and interacts with cargo molecules, and the appendages recruit accessory proteins for vesicle formation.

In their previous study, McMahon and colleagues found that α-appendages have two distinct interaction sites,
Unique Development in Hemichordates Suggests Some Unique Features of Chordates

Richard Robinson | DOI: 10.1371/journal.pbio.0040288

Underlying all the rich variety of form among chordates, from snakes to humans, are several invariant characteristics in body plan. One of the most fundamental of these is the front-to-back, or dorsal–ventral, axis. Our nerve chords run dorsally; our mouths project ventrally. This three-dimensional pattern in the adult is created by a four-dimensional pattern of gene expression during development, as transcription factors turn on and turn off suites of genes in concert.

Many of these transcription factors are even more ancient than the origin of our body plan, and are shared with other creatures, including arthropods, which also have bilateral symmetry and a central nervous system. In a new study, Christopher Lowe, John Gerhart, Marc Kirschner and colleagues show that many of these same signals are employed by the hemichordates, which are the phylum of bilaterally symmetrical adults closest to chordates but surprisingly do not have a central nervous system. However, the developing hemichordate interprets these signals in some ways that are significantly different both from chordates, which they are more closely related to, and arthropods, with which they nonetheless share some important features.

In both chordates and Drosophila, the canonical arthropod of the world of research, the dorsal–ventral axis develops in response to opposing gradients of two sets of proteins, Chordin and Bmp. In the embryo, where Chordin is high and Bmp is low, the nervous system develops (on the dorsal side for chordates; on the ventral side for arthropods). Nervous system development proceeds in two phases, both in response to Bmp gradients. First, the ectoderm (one of the three basic tissue layers in the embryo) segregates into epidermis (high Bmp) and neural tissue (low Bmp). Then, within the neural tissue, regions of high Bmp give rise to sensory neurons, while areas of low Bmp give rise to motor neurons and interneurons. (Bmp gradients also influence development of other organ systems in the other tissue layers.)

The acorn worm, Saccoglossus kowalevskii, is a hemichordate that lives in intertidal zones and grows to about 8 inches long. It is dorsoventrally polarized in the development and location of its organs, such as the gill slits, the gonads, and the heart/
The Path to Digestion Is Paved with Repair

Jason Underwood | DOI: 10.1371/journal.pbio.0040307

During the normal course of digesting a human meal, the stomach and subsequent meters of intestinal lining can sustain scratches and physical stresses as food winds through the coiled path. Abrasions are kept to a minimum through the activity of specialized cells that secrete mucus to lubricate the lining.

Now, a study by Katsuya Miyake, Toru Tanaka, and Paul McNeil suggests that the digestive track responds to stresses with a local lubrication response. They used a variety of mucus-producing rodent cells and tissues in combination with several damaging treatment methods to demonstrate that mucus is secreted at the site of injury. At the same time, cells repair their own damaged outer membrane by depositing a "patch" on the injury.

The authors used a simple, yet powerful approach to visualize mucus secretion. Mucus contains glycoproteins, which are modified protein–carbohydrate complexes. Glycoproteins can be monitored using fluorescent versions of proteins called lectins. Since these proteins bind tightly to carbohydrates, the location and intensity of the mucus can be inferred by monitoring the fluorescent glow under a microscope. They also developed an assay to carefully quantify how much mucus was secreted.

Miyake et al. grew gastric surface cells from a rat in culture and subjected them to a variety of stresses. As a general stress, they pushed the cells through a thin syringe needle multiple times, creating perforations in the plasma membrane. The assay revealed that the amount of mucus in the extracellular space increased in a remarkably linear fashion with the number of syringe strokes. Interestingly, without extracellular calcium, mucus secretion was absent. This hinted that the mucus response requires some form of calcium signaling.

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A surface mucous cell bordering on the stomach lumen secretes mucus (pink stain).
These observations led to an intriguing question: do cells respond to injury by switching on generalized secretion and repair or instead have a more specialized mechanism for localizing the mucus response and repairing the wound? To address this question, the researchers used a laser to cause targeted injuries to cells. The response was then visualized with a fluorescent lectin to monitor mucus levels while a special dye in the media monitored the repair response. Without a hole in the cell, the dye is found only on the outside of the cell. If a hole is formed by the laser and is not resealed, the dye can leak through the wound, resulting in a bright intracellular glow.

When the experiment was performed with calcium present, the laser insult resulted in a fast, potent response to the injury site. Mucus is preferentially secreted on the side of the cell where the injury occurred. Also, with calcium present, very little dye accumulates inside the cell during the experimental time course, indicating that the hole is quickly patched. Without extracellular calcium, mucus secretion is absent and the inside of the cell quickly fills with the dye. Without a hole in the cell, the dye is found only on the outside of the cell. If a hole is formed by the laser and is not resealed, the dye can leak through the wound, resulting in a bright intracellular glow.

The researchers’ time-lapse movies of these events (see DOI: 10.1371/journal.pbio.0040276.sv002) dramatically illustrate this point. Of course, injuries to the mammalian intestinal lining do not result from shear force or lasers, so Miyake et al. approximated the real situation with a different method: using a needle to scratch monolayer-cultured cells or segments of rodent colon. The same localized response was observed; mucus was secreted at the site of injury and resealing occurred. Again, calcium played a key role in both processes. When the authors examined the injury with electron microscopy, they could see that the extracellular membrane architecture changes drastically near the damaged site. Cells form new projections, as if a rough scab is laid down after injury. Again, they uncovered calcium as an essential partner to the repair machinery.

These results show that the cells of the stomach and intestine have an efficient mechanism for repairing ongoing assaults on the digestive tract. The injury itself acts as a signal for both mucus release and an emergency patch response. This adds another checkmark on the growing list of cellular events that function through calcium signaling. With this elegant mechanism in hand, the road ahead is full of important questions: how might one’s food intake, genetic disposition or an illness tweak the repair process? And what cellular proteins act as gatekeepers for this process? In any case, the normally dark digestive system has seen a new light.

Miyake K, Tanaka T, McNeil PL (2006) Disruption-induced mucus secretion: Repair and protection. DOI: 10.1371/journal.pbio.0040276

Evolution of Neonatal Imitation

Liza Gross | DOI: 10.1371/journal.pbio.0040311

Humans do it. Chimps do it. Why shouldn’t monkeys do it, too? Mimicry exists throughout the animal kingdom, but imitation with a purpose—matching one’s behavior to others’ as a form of social learning—has been seen only in great apes. (Mockingbirds can imitate an impressive number of other birds’ songs, but they can’t mimic you sticking out your tongue like a chimp can.) This matching behavior likely helps individuals conform to social norms and perform actions in the proper context. It’s generally believed that monkeys do not imitate in this way. However, the discovery that rhesus monkeys have “mirror neurons”—neurons that fire both when monkeys watch another animal perform an action and when they perform the same action—suggests they possess the common neural framework for perception and action that is associated with imitation.

Most studies exploring the early signs of matching behavior have focused on humans. A landmark 1977 study by Andrew Meltzoff and Keith Moore showed that 12- to 21-day-old infants could imitate adults who pursed their lips, stuck out their tongue, opened their mouth, and extended their fingers. They later found similar results in newborns, demonstrating that imitation is innate, not learned. A handful of studies on newborn chimps found a similar capacity for imitating human facial gestures. In a new study, Pier Ferrari, Stephen Suomi, and colleagues explored the possibility that imitation evolved earlier in the primate tree by studying neonatal imitation in rhesus monkeys, which split from the human lineage about 25 million years ago. They found that rhesus infants can indeed imitate a subset of human facial gestures—gestures the monkeys use to communicate. The first investigation of neonatal imitation outside the great ape lineage, their study suggests that the trait is not unique to great apes after all.

Ferrari et al. tested 21 baby rhesus monkeys’ response to various experimental conditions at different ages (one, three, seven, and 14 days old). Infants were held in front of a researcher who began with a passive expression (the baseline condition) and then made one of several gestures, including tongue protrusion, mouth opening, lip smacking, and hand opening.

Day-old infants rarely displayed mouth opening behavior, but smacked their lips frequently. When experimenters performed the mouth

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A newborn macaque imitates tongue protrusion.
opening gesture, infants responded with increased lip smacking but did not increase any other behavior. None of the other stimuli produced significant responses. But by day 3, matched behaviors emerged: infants stuck out their tongues far more often in response to researchers’ tongue protrusions compared with control conditions, and smacked their lips far more often while watching researchers smacking theirs. (Watch an infant imitating mouth opening at DOI: 10.1371/journal.pbio.0040302.sv001.) By day 7, the monkeys tended to decrease lip smacking when humans performed the gesture, and by two weeks, all imitative behavior stopped.

Infant rhesus monkeys, these results suggest, have a narrow imitation window that opens three days after birth, when they can reproduce human tongue protrusion and lip smacking. This imitation period is much longer in humans (two to three months) and chimps (about two months).

It’s possible that rhesus babies show more varied and prolonged imitative behavior in response to mom or other monkeys than to human experimenters, who may not provide the most relevant biological cues. But this narrow window does comport with the development schedule of rhesus monkeys, which is much shorter than that of humans and chimps.

Many questions remain about the neural mechanisms of neonatal imitation. The researchers argue that their results support a resonance mechanism linked to mirror neurons, which have recently been identified while monkeys observe others’ lip smacking and tongue protrusion. In this model, observing human mouth gestures directly activates mirror neurons in the monkeys’ brain, ultimately leading to a replication of the gesture.

Human babies can imitate an adult’s facial gesture a day after seeing it, which may help them identify individuals. For rhesus monkeys, lip smacking (which often alternates with tongue protrusion) accompanies grooming sessions and signals affiliation—an important social cue for a species that is often described as “despotic and nepotistic.” Picking up these social gestures early in life may well facilitate the animal’s early social relations (primarily with the mother) and assimilation into the social fabric of the group, providing a mechanism for distinguishing friend from foe. It will be interesting to test the extent of imitation in monkeys with more complex social dynamics. While the social life of rhesus monkeys may not demand the more sophisticated repertoire of behaviors seen in great apes, they seem to be hard-wired for imitation just like apes.

Ferrari PF, Visalberghi E, Paukner A, Fogassi L, Ruggiero A, et al. (2006) Neonatal imitation in rhesus macaques. DOI: 10.1371/journal.pbio.0040302