

## Synopsis of Research Articles

## Ciliate Genome Sequence Reveals Unique Features of a Model Eukaryote

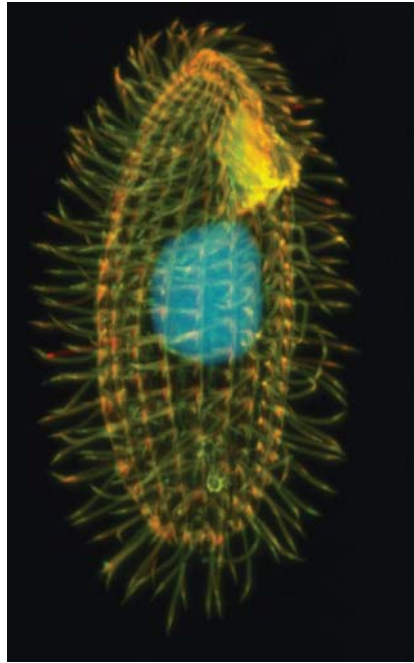
Richard Robinson | DOI: 10.1371/journal.pbio.0040304

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 *Paramecium*, 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



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**The genome sequence of the single-celled ciliate *Tetrahymena thermophila* sheds light on early eukaryotic evolution.**

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 micronucleus 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

# Multiple Pathways Give a No-Frills Nervous System a Flexible Oxygen Response

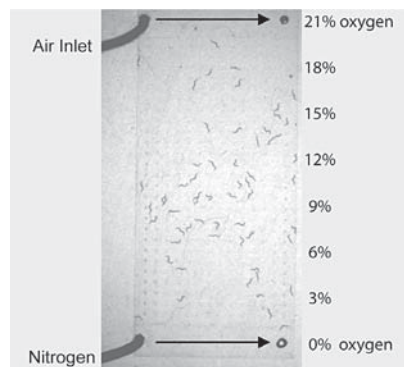
Mary Hoff | DOI: 10.1371/journal.pbio.0040306

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



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## Wild-type *C. elegans* prefers 7%–14% oxygen when placed in an oxygen gradient.

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.0040274

## 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.

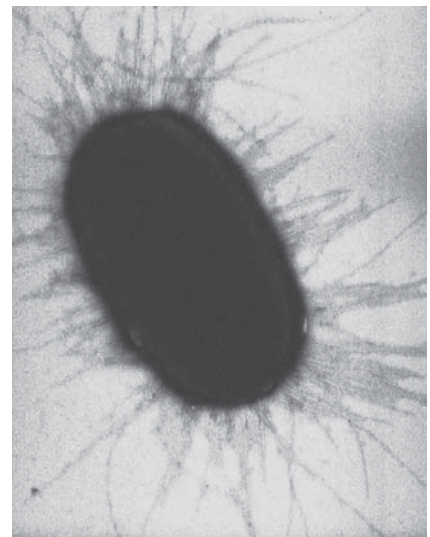
Fimbriae-mediated adhesion was investigated with an atomic force microscope, which uses a cantilever to apply (and measure) forces between its tip and the sample under investigation. Forero et al. outfitted the cantilever tip with mannose, and then used this to touch a fimbriated *E. coli* cell that

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, coiling forces are too weak to switch bonds to a long-lived state before breaking, and higher, uncoiling forces



DOI: 10.1371/journal.pbio.0040314.g001

**The long, sticky filaments covering *E. coli* bacteria uncoil under force, apparently improving the binding of the terminal adhesive unit in the presence of forces generated by fluid flow. (Image: Manu Forero)**

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**

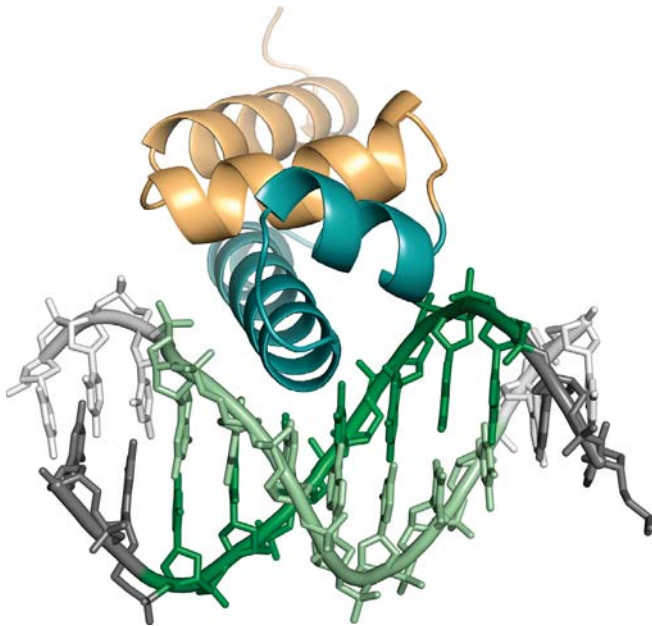
## A Bacterial Protein Puts a New Twist on DNA Transcription

Mason Inman | DOI: 10.1371/journal.pbio.0040294

For organisms to adapt, develop, and simply live, they must regulate hundreds to thousands of genes, making fine-tuned, precisely timed adjustments to produce the specific complement of proteins required for the occasion. For bacteria, this task falls largely to proteins called sigma factors. These small proteins associate with RNA polymerase, the enzyme that mediates gene transcription, to form a complex called the holoenzyme. The holoenzyme, guided by the sigma factor, recognizes promoter regions, which are specific DNA sequences that precede protein-coding sequences and mark the transcription start site. Sigma factors also contribute to

transcription by facilitating DNA strand separation, which must occur before RNA polymerase can begin copying the DNA code. Once transcription begins, the sigma factor disengages from the RNA polymerase, becoming available for new joint ventures with different RNA polymerases.

A single sigma factor can control the expression of hundreds of genes through these partnerships, carrying out everything from basic metabolic activities to physiological responses to environmental stress (which, for bacteria, might include antibiotic therapy). Knowing how sigma factors bind to DNA is an important step in understanding how they



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**A crystal structure of domain 4 of an *E. coli* Group IV sigma factor bound to -35 element DNA identifies a unique interaction mechanism that relies on the rigid conformation of the DNA consensus sequence.**

mediate their cosmopolitan regulatory duties. Structural studies provide important clues to the nature and function of associations between sigma factors and DNA. In a new study, William Lane and Seth Darst used structural analysis techniques to determine the detailed shape of one type of sigma factor. They show that it binds to short DNA sequences using a molecular recognition method that has not been seen before in sigma factors.

Sigma factors come in two structurally unrelated families: sigma 54 and sigma 70. The sigma 54 family is associated with a diverse range of metabolic processes. The much larger sigma 70 family encompasses four groups: the Group I “primary” sigma factors facilitate metabolic and growth processes; the Group II–IV “alternative” sigma factors mediate specialized processes like sporulation and the environmental stress response. The sigma 70-type sigma factors recruit the RNA polymerase holoenzyme to bipartite

promoter sequences, comprising conserved sequence elements centered about 10 and 35 base pairs upstream of the transcription start site. These so-called -10 and -35 elements are recognized by distinct structural domains of the sigma factor. Structures of one of the most studied sigma factors, a primary sigma factor called sigma-A, have been solved in previous studies. Here, Lane and Darst analyzed the -35-element-binding domain (domain 4) of an alternative Group IV sigma factor found in *Escherichia coli*, called sigma E4. Group IV sigma factors comprise the largest and most diverse set of sigma factors.

Both sigma-A4 and sigma-E4 allow RNA polymerase to bind to the -35 promoter element, but in each case the sequence is very different. In the case of sigma-E4, the sequence is GGAAGTT (and others that resemble it). Previous studies showed that sigma-A4 recognizes its consensus sequence, TTGACA, through direct interactions with these six nucleotide bases. It was tempting to assume that sigma-E4 would operate in a similar manner, since the two sigma factors are similar in structure.

But, using X-ray crystallography, Lane and Darst showed that sigma-E4 binds its consensus sequence using a more subtle method. By determining the structure of the sigma factor bound to its consensus sequence, they found that sigma-E4 doesn’t recognize the identity of the sequences per se but the shape of the DNA helix at those sequences. While one region of the sigma factor sits deep within a groove along the double helix’s side, another region holds the promoter -35 sequence straight. The AA in the center of sigma-E4’s consensus sequence, the researchers believe, is required for the DNA to assume this shape.

Because evolution has conserved the site in these proteins that sits alongside the AA of the consensus sequence, Lane and Darst propose that this method of recognizing -35 promoter sequences may be common across the Group IV sigma factors. With further studies of the structures of sigma factors and their means of recognizing specific promoters—and thus activating specific genes—researchers can better predict the full complement of genes a given promoter will regulate, and in turn gain insight into the diverse physiological responses they help mediate.

Lane WJ, Darst SA (2006) The structural basis for promoter -35 element recognition by the group IV sigma factors. DOI: 10.1371/journal.pbio.0040269

## Anatomy of a Fever

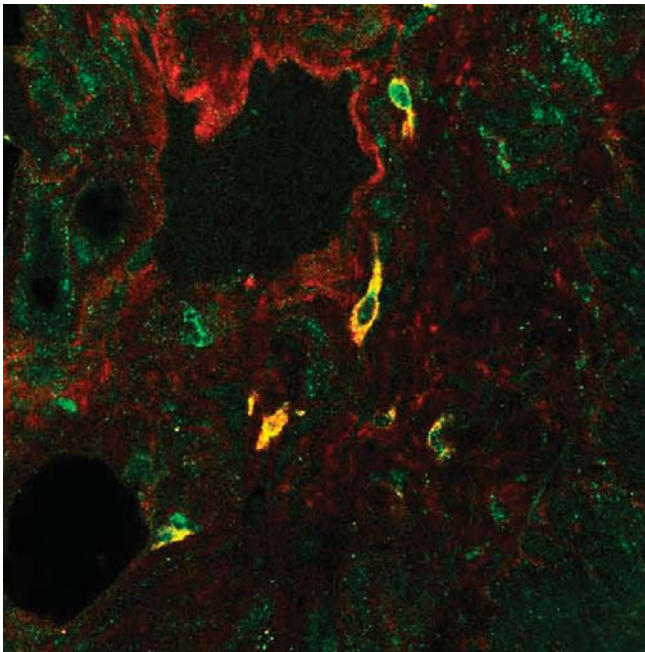
Liza Gross | DOI: 10.1371/journal.pbio.0040305

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 E<sub>2</sub> (PGE<sub>2</sub>), can pass through multiple temperature phases.

While it’s well established that PGE<sub>2</sub> 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 PGE<sub>2</sub> 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 PGE<sub>2</sub> synthesis doesn’t begin in the brain as previously thought, but in the lungs and liver. They also describe the molecular mechanisms that produce PGE<sub>2</sub> in these organs.

Many of the mechanisms of fever have been established by exposing rodents to bacterial endotoxins called



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**Cyclooxygenase-2 (green immunofluorescence) and the macrophage marker ED2 (red immunofluorescence) in rat lung at the onset of bacterial lipopolysaccharide-induced fever (photo: Jordi Serrats).**

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<sub>2</sub> synthesis. Thus, fever starts about an hour before the PGE<sub>2</sub>-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<sub>2</sub> produced peripherally, outside the brain.

To test this hypothesis, Steiner et al. gave rats an intravenous (IV) solution of PGE<sub>2</sub> bound to albumin, the

primary transporter of PGE<sub>2</sub> in the blood. Controls received an IV albumin solution. After confirming that the PGE<sub>2</sub> infusion induced fever, the researchers collected venous and arterial blood from LPS-treated rats. (PGE<sub>2</sub> synthesized in tissues amasses in venous blood; arterial blood delivers PGE<sub>2</sub> to the brain.) At the onset of fever, PGE<sub>2</sub> was significantly elevated both in the venous and arterial blood.

To investigate the origin of fever-inducing PGE<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub>.

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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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**

## Master Proteins Dictate Retinal Differentiation Timetable

Liza Gross | DOI: 10.1371/journal.pbio.0040293

The embryonic construction of the vertebrate retina is a highly ordered affair. Following a precise timetable, six different specialized cell types emerge from a mass of identical, proliferating cells. The process of retinal cell differentiation, when so-called progenitor cells stop dividing and choose among the six fates, depends primarily on homeobox genes, major regulators of embryonic patterning. How these genes control the timing of retinal cell differentiation has remained an open question—until now.

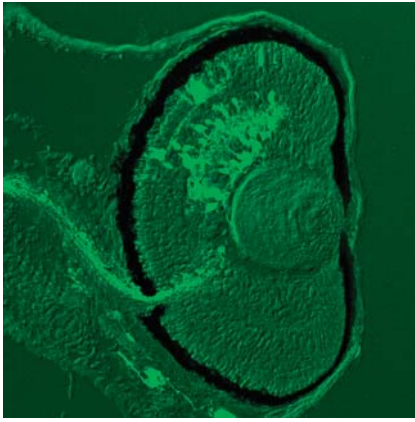
In a new study, Sarah Decembrini, Federico Cremisi, and colleagues

show that three homeobox genes work in conjunction with a cellular timepiece that determines the sequential emergence of distinct cell types. Surprisingly, the schedule of both homeobox gene expression and retinal cell differentiation is controlled by the translation, rather than by the transcription, of the genes.

Retinal cells transform light signals into visual information for further processing in the brain. After light stimulates the rod and cone photoreceptors, visual signals travel to horizontal and bipolar cells, which in turn interface with amacrine cells. Ganglion cells, which then relay these

signals to the brain, are the first-born cells—that is, the first to exit the cell cycle and stop dividing. Though their birthdays vary somewhat by species, the horizontal, cone, and amacrine cells come next, then the rod and bipolar cells.

Decembrini et al. suspected that cell identity may be tied to cell cycle progression because different retinal cell types are produced when cell cycle length is manipulated. To test this hypothesis, they studied a subset of homeobox genes, including *otx5*, which supports photoreceptor differentiation, and *vsx1* and *otx2*, which promote bipolar differentiation.



DOI: 10.1371/journal.pbio.0040293.g001

**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 *Xotr2* 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.

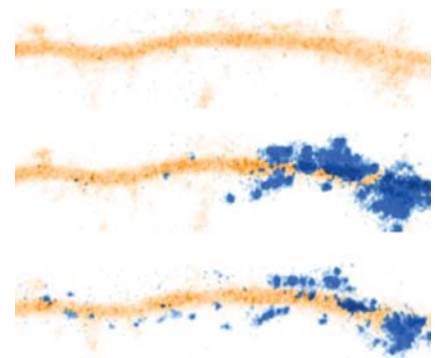
Altogether, these results indicate that a post-transcriptional mechanism regulates when these proteins are expressed and in which cells. This mechanism operates in synch with a cellular clock that measures cell cycle length to generate the later developing photoreceptors and bipolar cells. The next step 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, Andreatzoli M, Vignali R, Barsacchi G, Cremisi F (2006) Timing of the generation of distinct retinal cells by homeobox proteins. DOI: 10.1371/journal.pbio.0040272

## 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 constantly move 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 Tsuruel, 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.



DOI: 10.1371/journal.pbio.0040297.g001

**Photoactivation of synapses on one side of a dendritic segment (orange) is followed by migration and incorporation of photoactivated PA-GFP-tagged ProSAP2 (blue) into neighboring synapses.**

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

at synapses, including synaptic vesicles chock-full of neurotransmitters on the axonal (presynaptic) side, and neurotransmitter receptors on the dendritic (postsynaptic) side. Synapsin I and ProSAP2 play important structural roles: Synapsin I tethers synaptic vesicles underneath the presynaptic lipid membrane and ProSAP2 organizes the postsynaptic architecture.

To follow the whereabouts of Synapsin I and ProSAP2, the researchers tagged each protein with fluorescent dyes and coaxed cultured neurons from the hippocampus (a brain region involved in learning) of newborn rats to synthesize these fluorescently tagged proteins. As the neurons grew in culture, they established synapses that incorporated the tagged Synapsin I or ProSAP2. The synapses were easily visualized as bright fluorescent spots studding dendrites and axon branches. The first dye, called green fluorescent protein (GFP, a small protein that was originally isolated from jellyfish), fluoresces readily but can be extinguished with intense illumination, a phenomenon called photobleaching. The researchers photobleached individual synapses containing GFP-tagged Synapsin I or ProSAP2 with an intense laser

beam. Over time, a fluorescent signal reappeared at the bleached synapses, indicating that bleached proteins were replaced with tagged proteins from unbleached areas. Tagged Synapsin replenished bleached synapses in about 40 minutes, and tagged ProSAP2 in two to four hours.

But these experiments did not show where the replenishing proteins came from. To answer this question, the researchers took advantage of a second dye, photoactivatable variant of GFP (PA-GFP), whose fluorescence is activated, rather than extinguished, with intense illumination. The researchers photoactivated PA-GFP-tagged Synapsin I or ProSAP2 over small portions of dendrites or axons. Over the course of 10 to 40 minutes, fluorescence gradually declined at the illuminated synapses, and concomitantly increased in neighboring synapses. These results indicate that pre- and postsynaptic proteins routinely hop from one synapse to the next with timescales of tens of minutes, a behavior that might account for the rapid replenishment of photobleached synapses.

Still, some of the replenishing material could also have come from new protein synthesis. By tracking PA-GFP-tagged proteins from cell

bodies, where most synthesis typically occurs, into dendrites and axons, the researchers determined that newly synthesized Synapsin I and ProSAP2 moved too slowly to explain the rapid replenishment of bleached synapses. In addition, inhibitors of protein synthesis and degradation did not significantly affect the synapses' replenishment rates, confirming that the high turnover rate of Synapsin I and ProSAP2 owes mostly to local exchanges among neighboring synapses.

How the promiscuous exchange of structural proteins such as Synapsin I and ProSAP2 affects synaptic stability is still unclear. Competition for a local pool of synaptic components could eventually determine which synapse is stabilized. Curiously, synaptic signaling may be a destabilizing factor in the young hippocampal neurons, as electric stimulations to the cultures greatly increased Synapsin I and ProSAP2 trafficking. Whether local promiscuity is a characteristic of youthful synapses or also holds true for more mature ones remains to be seen.

Tsuriel S, Geva R, Zamorano P, Dresbach T, Boeckers T, et al. (2006) Local sharing as a predominant determinant of synaptic matrix molecular dynamics. DOI: 10.1371/journal.pbio.0040271

## Conflict within the Genome: Evolving Defenses to Suppress the Male Killers

Liza Gross | DOI: 10.1371/journal.pbio.0040308

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 counteracting 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 *wBo1* 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



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**A male nymphalid tropical butterfly *Hypolimnas bolina*, otherwise known as the Common Eggfly. (Photo: Sylvain Charlat)**

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 *wBoll* 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 *wBoll* genes (and infection) onto the butterfly genetic background (a technique called introgression).

The breeding experiments tested two questions: would male-killing *wBoll* taken from Moorea in Polynesia lose that ability against Southeast Asian males with a Thai or Philippine genetic background, and would benign *wBoll* 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, *wBoll*-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 *wBoll* 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 *wBoll* 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 *wBoll* 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 13 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

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

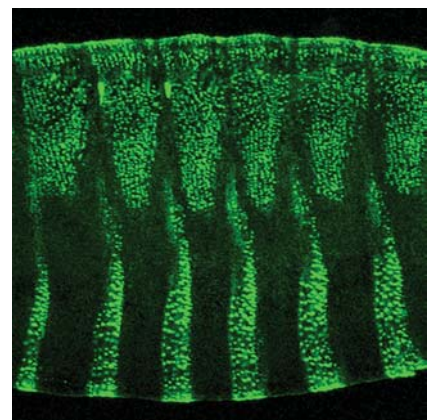
Richard Robinson | DOI: 10.1371/journal.pbio.0040310

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 H el ene 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



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**Cell shape remodeling: analyzing the function of genes specifically expressed in cellular extensions of *Drosophila* embryonic epidermis (green stain of a protein called *Miniature*).**

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 *miniature* 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 *miniature* gene, influencing its transcription.

But is *miniature* 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 *miniature* 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. *miniature*, 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 denticle 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.

**Chanut-Delalande H, Fernandes I, Roch F, Payre F, Plaza S (2006) Shavenbaby couples patterning to epidermal cell shape control. DOI: 10.1371/journal.pbio.0040290**

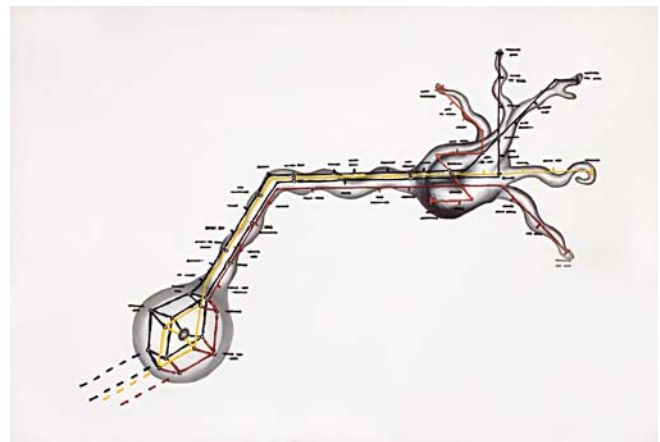
## Sharing Responsibility for Clathrin Coat Assembly

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 assembles 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



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**Painting by Alex McCuish (Hills Road Sixth Form College, Cambridge, United Kingdom) illustrating the protein interaction network underlying nerve function.**

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,  $\beta$ -appendage and  $\alpha$ -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  $\alpha$ -appendages have two distinct interaction sites,

allowing for clustered adaptor proteins to interact with many accessory proteins simultaneously. The AP2  $\alpha$ -appendage becomes a hub for protein interactions only in the initial stages of assembly. In this study, they focused on the  $\beta$ -appendage.

First, Schmid et al. determined the interaction partners of both appendages by removing the bound partners from cell extracts then analyzing them with mass spectrometry. They found a number of previously unidentified interaction partners for the  $\beta$ -appendage (and a few more for the  $\alpha$ -appendage). Some interact only with the  $\beta$ -appendage, but many also interact with the  $\alpha$ -appendage.

To understand the molecular details of the interactions, the researchers mutated key regions of the  $\beta$ -appendage interaction sites (the  $\beta$ -appendage also has a top and side site) then assessed the impact on their binding partners. They found that the top site mediates most interactions for the  $\alpha$ -appendage and the side site does the same for the  $\beta$ -appendage. With this setup, accessory proteins that bind to the  $\alpha$ -appendage's top site can also bind to the  $\beta$ -appendage's side site, leaving the appendages' other sites free to interact with still more proteins. Interactors can bind to multiple appendages, allowing APs to serve as scaffolds for protein assembly. These results do not fully explain why two appendages exist, the researchers acknowledge, but because the same proteins interact with the top and side sites,

it's likely that the appendages collaborate to mediate these interactions.

Clathrin coat formation, Schmid et al. propose, is an outgrowth of increasingly stable interactions among a shifting network of proteins. Rapidly shifting interactions between isolated proteins give rise to coordinated, dynamic interactions between a network of proteins centered around the membrane, then to increasingly stable interactions as the coat assembles. The presence of both activated cargo receptors and lipid signaling molecules (phosphoinositides) in the membrane trigger the accumulation of adaptor complexes, which rapidly stabilize with the help of accessory proteins with multiple sites for AP2 appendage interactions. The accessory proteins recruit clathrin, which interacts with  $\beta$ -appendages and displaces accessory proteins as it accumulates and self-assembles during coat formation. Accessory proteins that interact only with appendages are shunted to the side, where clathrin polymers have not yet formed, while accessory proteins that can interact with clathrin are maintained. Having demonstrated the power of using a multidisciplinary approach to study the endocytic interactome, the researchers believe that the principles uncovered will apply to other protein networks.

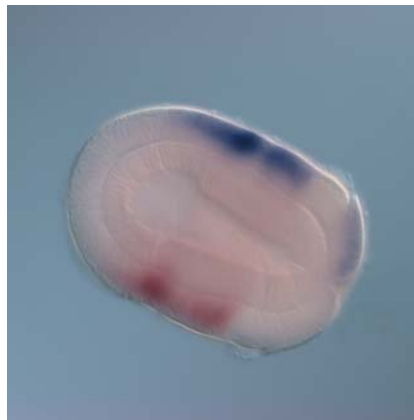
Schmid EM, Ford MGJ, Burtay A, Praefcke GJK, Peak-Chew SY, et al. (2006) Role of the AP2  $\beta$ -appendage hub in recruiting partners for clathrin-coated vesicle assembly. DOI: 10.1371/journal.pbio.0040262

## 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



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**The embryo of the hemichordate *Saccoglossus kowalevskii* expresses the *chordin* gene on the ventral midline and the *bmp2/4* gene on the dorsal midline. All dorsoventral development depends on this Bmp-Chordin polarity.**

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/

kidney complex. It has a nerve net and axon tracts, but no central nervous system—there is no brain-like mass of neural ganglia—and, unlike chordates and arthropods, its nerve cells and epidermal cells are finely intermixed.

The authors showed that Bmp and Chordin act within the hemichordate embryo to establish the dorsal–ventral axis, and they identified multiple genes along this axis whose expression was influenced by these two proteins. When they supplied an excess of Bmp, embryos became excessively dorsialized in their expression domains and anatomical features; the opposite occurred when Bmp was diminished. In this respect, the pattern was similar to arthropods and chordates. (Along with its theoretical importance, the identification of this molecular determinant of anatomy aids the practical study of the creature as well, since its anatomy, plus the fact that it spends most of its time in the vertical position, has made it difficult to unambiguously identify a dorsal or ventral side.)

Unlike in either chordates or *Drosophila*, the first phase of neurogenesis in the hemichordate embryo did not respond to changes in Bmp–Chordin concentration—there was no alteration in the neural ectoderm-versus-epidermis differentiation as Bmp was increased. These results suggest that the Bmp–Chordin-mediated centralization of the nervous system, occurring in both arthropods and chordates, arose independently in the two groups after they diverged. The Bmp–Chordin gradient did affect differentiation of neuronal cell types in the hemichordate embryo, analogous to the second phase of patterning in chordates and *Drosophila*, although the details differed significantly. These differences suggest that much of the “regulatory architecture” of the developing nervous system evolved after the chordate–hemichordate split. The full implications of these differences remain to be worked out.

Finally, the results from this study shed light on an important question

in comparative evolutionary anatomy. While high Chordin and low Bmp characterize the dorsal side of the chordate embryo, this pattern is associated with the ventral (mouth) side of the *Drosophila* embryo, as well as in all other bilaterally symmetric animals, including the hemichordates. This suggests that during early chordate evolution there was an inversion in the Bmp–Chordin developmental axis relative to the mouth, by movement of the mouth or axis, resulting in the chordate’s unique pattern of response to these ubiquitous molecular determinants. Before or after this inversion, the nervous system was centralized to the Chordin (low Bmp) side in the chordate lineage. Further study is needed to answer these questions.

Lowe CJ, Terasaki M, Wu M, Freeman RM Jr, Runft L, et al. (2006) Dorsoventral patterning in hemichordates: Insights into early chordate evolution. DOI: 10.1371/journal.pbio.0040291

## The Path to Digestion Is Paved with Repair

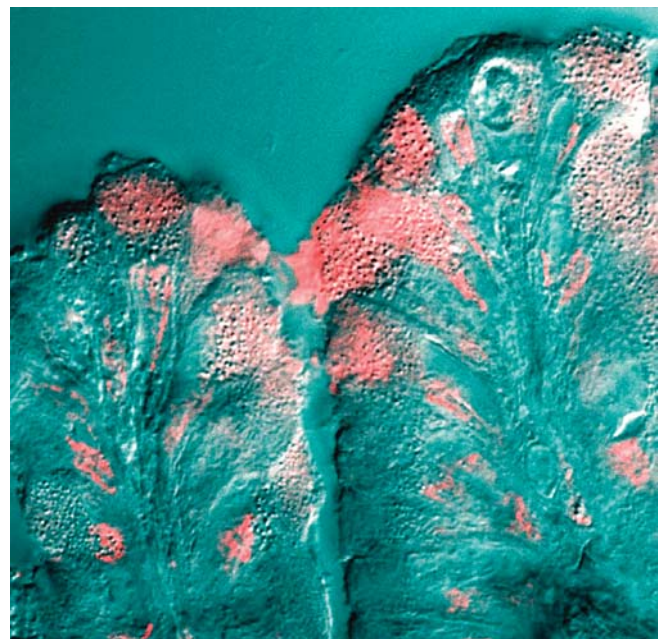
Jason Underwood | DOI: 10.1371/journal.pbio.0040307

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



DOI: 10.1371/journal.pbio.0040307.g001

**A surface mucous cell bordering on the stomach lumen secretes mucus (pink stain).**

number of syringe strokes. Interestingly, without extracellular calcium, mucus secretion was absent. This hinted that the mucus response requires some form of calcium signaling.

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. The researchers' time-lapse movies of these events (see DOI: 10.1371/journal.pbio.0040276.sv001 and 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.



DOI: 10.1371/journal.pbio.0040311.g001

### A newborn macaque imitates tongue protrusion.

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

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**