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 macronuclear 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 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(1f)* 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 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...
Sigma factors also contribute to sequences that precede protein-coding sequences and mark enzyme that mediates gene transcription, to form a complex. These small proteins associate with RNA polymerase, the bacteria, this task falls largely to proteins called sigma factors. For organisms to adapt, develop, and simply live, they must 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

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 uncoiling 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

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)

DOI: 10.1371/journal.pbio.0040314.g001

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
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 GGAACCTT (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 s 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 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...
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 otx3, which supports photoreceptor differentiation, and vsx1 and otx2, which promote bipolar differentiation.
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 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.
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
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 a*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 a*Bol1* genes (and infection) onto the butterfly genetic background (a technique called introgression).

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

**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
*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.

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
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

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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).

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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. 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.

Evolution of Neonatal Imitation

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
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