

Synopsis of Research Articles

Simple Peptides Stabilize Mighty Membrane Proteins for Study

DOI: 10.1371/journal.pbio.0030259

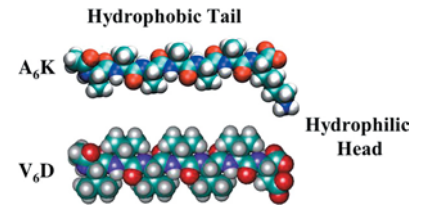
Cell membranes are largely made of proteins, and membrane proteins account for about a third of all genes. Despite their importance, they are devilishly hard to isolate and stabilize, and therefore are hard to study. The problem lies in their structure: membrane proteins have at least one hydrophobic domain, composed of a stretch of water-repelling amino acids, which holds the protein snugly in the lipid membrane. Purifying such a protein in an aqueous medium makes the hydrophobic parts aggregate, destroying the protein's delicate three-dimensional structure and often disrupting its function. The alternative is to extract the protein with a detergent, a two-headed "Janus" molecule with both hydrophobic and hydrophilic ends. The protein remains surrounded by the hydrophobic ends, while water clusters at the hydrophilic ends, easing the protein out of the membrane and into solution, where it can be studied.

To date, though, relatively few complex membrane proteins have been successfully purified with available detergents. In this issue, Shuguang Zhang and colleagues show that a simple amino acid-based detergent can successfully

stabilize the dauntingly large protein complex photosystem I (PS-I), an integral part of the photosynthetic machinery.

The molecule they made, abbreviated A_6K , links six units of the hydrophobic amino acid alanine to one of the hydrophilic amino acid lysine. The authors used it to stabilize PS-I and then attached the detergent-protein complex to a glass slide, allowed it to dry, and examined the stability of PS-I by testing its fluorescence. Intact PS-I emits red light with a characteristic peak wavelength; as it degrades, this peak subsides and is replaced by another, bluer peak. Even the two best standard detergents did poorly at maintaining the red peak. In contrast, the spectrum after A_6K extraction was almost a perfect match for the normal one, indicating the complex was largely intact after drying. Furthermore, the complex appeared to remain stable for up to three weeks on the glass slide.

The potential applications of this work are severalfold. PS-I itself remains to be fully characterized, and this stabilization technique offers new means to explore its properties. In addition, an isolated and stabilized form of PS-I may hold some promise as an alternative energy source,



DOI: 10.1371/journal.pbio.0030259.g001

The designed short peptide (protein fragment) detergents look like matches and behave like lipids or oil molecules that repel water at one end but attract water at the other end

since it generates an electric current in sunlight. Perhaps most importantly, the full potential of such simple amino acid-based detergents has only begun to be explored. It is likely that either this one, or others like it, can be used to isolate and stabilize hundreds of other membrane proteins, allowing them to be studied in detail for the first time.

Kiley P, Zhao X, Vaughn M, Baldo MA, Bruce BD, et al. (2005) Self-assembling peptide detergents stabilize isolated photosystem I on a dry surface for an extended time. DOI: 10.1371/journal.pbio.0030230

miRNA Processing: Dicer-1 Meets Its Match

DOI: 10.1371/journal.pbio.0030244

In recent years, the control of gene expression by small RNA molecules has emerged as a major new mechanism for gene regulation. The small RNAs interfere with the expression of their target gene by reducing its transcription, triggering the destruction of the gene transcript, or inhibiting its translation into a protein. This discovery has not only altered views of gene regulation, but also provided molecular geneticists with powerful new tools with which to study and manipulate the function of any gene. The biology of these small RNAs is, therefore, under intense scrutiny.

Small RNAs are generated by specific pathways, the elements of which are being rapidly discovered. In this issue of *PLoS Biology*, two groups have identified a missing piece in one such pathway—in the fruitfly *Drosophila*. The pathway under investigation leads to the production of a type of small RNA called a microRNA (miRNA). These are 21–23 nucleotides in length, and are involved in regulating the expression of many genes. miRNAs start life as a much bigger transcript called a pri-miRNA, which is processed in two steps. First, it is converted into a shorter pre-miRNA, by the action of two proteins: Drosha, an RNase III enzyme; and Pasha, which contains double-stranded RNA binding domains (dsRBDs). The pre-miRNA is then transported to the cytoplasm and is trimmed again into a

double-stranded miRNA by a different RNase III enzyme called Dicer-1.

In a separate pathway, RNAs called small interfering RNAs (siRNAs) depend on the Dicer-2 RNase III and a dsRBD protein called R2D2 for their function. These pathways are also conserved in other organisms. Thus, a pattern emerges: the functions of small RNAs tend to require the combined actions of an RNase III and a dsRBD protein. But why doesn't Dicer-1 have a partner? The answer, provided by the two studies from the labs of Phil Zamore and Haruhiko and Mikiko Siomi, is that we just hadn't found it yet.

The two groups took different approaches to finding Dicer-1's partner. Zamore's group looked for genes resembling other dsRBD-encoding genes, while the Siomi lab did a functional screen for new genes specifically implicated in miRNA processing. They both homed in on a new gene with great similarity to R2D2, and showed that loss of function of the gene results in the accumulation of pre-miRNAs—very similar to loss of Dicer-1 function, which suggests that the two genes act together in the same pathway. The new potential partner of Dicer-1 was given the name *loquacious* (*loqs*), because failure to process the miRNAs in turn causes increased levels of expression of the target genes for the miRNAs.

Both groups also show that Loqs and Dicer-1 exist in a complex within the cell, and that the complex is able to process pre-miRNA into its mature form. The Siomis' lab went on to show that the complex contains a protein called Ago-1, which hints that the complex might also be involved in the action of miRNAs on their target genes, as well as in miRNA processing itself. Both groups also point out the similarity between Loqs and a human dsRBD protein called TRBP, which has been implicated in the response to infection by HIV.

There seems little doubt, then, that Dicer-1's partner has been found, and that the combined action of an RNase III and a dsRBD protein is a consistent theme in the function of miRNAs and siRNAs. The identification of Loqs will help to refine our

views of how miRNAs are processed, as well as how they can be manipulated. The connections made with processes such as stem cell maintenance (identified by the Zamore lab) and viral infection in these new studies also emphasize that gene regulation by small RNAs is relevant to a broad range of cellular physiology.

Förstemann K, Tomari Y, Du T, Vagin VV, Denli AM, et al. (2005) Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. DOI: 10.1371/journal.pbio.0030236

Saito K, Ishizuka A, Siomi H, Siomi MC (2005) Processing of pre-microRNAs by the Dicer-1–Loquacious complex in *Drosophila* cells. DOI: 10.1371/journal.pbio.0030235

Drosophila Larval Development and Human Immunodeficiency: The Adenosine Deaminase Connection

10.1371/journal.pbio.0030232

For most healthy individuals, infection triggers a rapid immune response that repels the invaders. But for those rare individuals born without the immune system cells (lymphocytes) that recognize and kill pathogens, bacterial, viral, or fungal encounters can result in recurrent infections that are more life-threatening and less responsive to treatment than similar infections in normal infants. In the past, all that could be done for children with severe combined immunodeficiency (SCID) was to protect them from infections by cocooning them in sterile plastic bubbles, which gave the disease its common name: bubble-boy syndrome. Nowadays, the treatment of choice, provided a suitable donor is available, is bone-marrow or stem-cell transplantation, which provides SCID children with a functioning immune system.

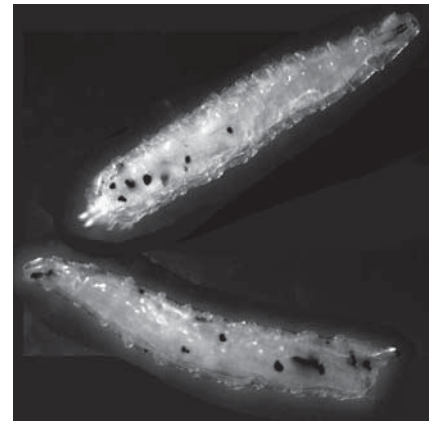
Mutations in at least nine genes can cause human SCID, but 20% of cases are caused by a deficiency of the enzyme adenosine deaminase. This enzyme, which is present in all organisms, converts adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. When adenosine deaminase is missing, its substrates (adenosine and deoxyadenosine) accumulate, and this is thought to cause the complete breakdown in immune defense characteristic of SCID.

To explore the role of adenosine deaminase in a tractable model system, Peter Bryant and his colleagues have now developed a *Drosophila* model by disabling the expression of a protein—called adenosine deaminase-related growth factor A (ADGF-A)—that serves as a major adenosine deaminase in the fly. In flies lacking ADGF-A enzymatic activity, adenosine and deoxyadenosine

concentrations increase in the larval hemolymph, the circulatory fluid or “blood” of insects. Lack of the enzyme, the researchers report, caused larval death associated with the disintegration of the fat body (the adipose tissue spread throughout the body of the insect), melanotic tumors, and delays and defects in development.

The first two effects, fat body disintegration and the presence of melanotic tumors, are directly attributable to dysregulation of hemocytes (fly blood cells) in the mutant animals. It turns out that in *adgf-a* mutant larvae, hemocytes are released prematurely from the lymph glands (the organs where hemocytes are produced and stored). These prematurely released hemocytes then cause fat body disintegration and formation of melanotic tumors; however, if ADGF-A expression is selectively restored in the lymph glands, then hemocytes are not prematurely released, and the larvae survive and develop without the tumors or fat body disintegration.

Bryant and his colleagues reasoned that the elevated adenosine might have direct effects on fly development aside from the dysregulation of hemocytes, so they examined the development of *adgf-a* mutant flies that also lacked a functional adenosine receptor (*adoR* mutants). They found that *adgf-a/adoR* mutant larvae were able to survive and continue development to adulthood, although these animals still experienced fat body disintegration and some melanotic tumors. These results suggest that the second consequence of ADGF-A deficiency, delayed development, is caused by the elevated adenosine in the animals signaling through adenosine receptors.



DOI: 10.1371/journal.pbio.0030232.g001

Drosophila adgf-a mutant larvae with melanotic tumors in their body cavities

Altogether, these results establish *adgf-a* flies as a useful model system for unraveling the many effects that adenosine and deoxyadenosine have on cellular physiology in general and on the immune system in particular. Because hemocyte release from lymph glands and delays in development also occur in response to infection, the authors hypothesize that adenosine might be involved in controlling hemocyte release and postponing development when fly larvae are challenged by microbial attacks. Future experiments in this model system should provide important clues to the pathology of adenosine deaminase deficiency-associated SCID and should also advance our understanding of how adenosine acts as a stress hormone during infections in individuals with normal immune systems.

Dolezal T, Dolezelova E, Zurovec M, Bryant PJ (2005) A role for adenosine deaminase in *Drosophila* larval development. DOI: 10.1371/journal.pbio.0030201

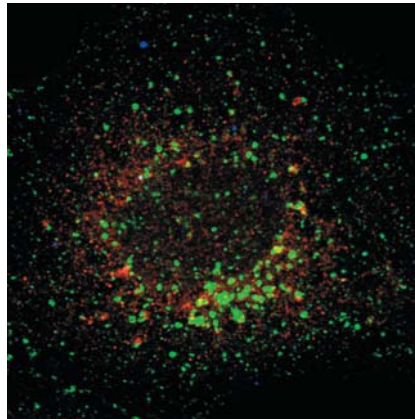
Sorting and Transporting a Viral Cargo: The Role of the Rab7 Protein

DOI: 10.1371/journal.pbio.0030260

When Robert Hooke first looked at cork bark with a light microscope in 1655, he saw small empty chambers, reminiscent of monastery cells. We now know that living cells are full of organelles—specialized subcompartments surrounded by membranes in which different cellular life functions occur. This complex organization raises major transport and sorting problems similar to those encountered in a large city in which trains and trucks carrying different cargos arrive at peripheral distribution centers. The cargos must be sorted and transported to individual factories where goods are made for delivery to other city destinations or for export. At the same time, the different areas of the city produce waste products that also need to be sorted and transported correctly. Somehow, thousands of cargos must end up in exactly the right place in both the city and the cell.

One cellular system that sorts and transports cargos is the clathrin-mediated endocytic pathway. Endocytosis—the ingestion of materials into the cell—is important for the interaction of cells with the environment because it allows the uptake of nutrients (the equivalent of the raw materials brought into the city) and signaling molecules (the letters brought in by the mail service). In clathrin-mediated endocytosis, materials arriving at the outside surface of the cell are engulfed in special areas of membrane known as coated pits, which pinch off to form intracellular vesicles. These lose their clathrin coat and other molecules involved in their formation to become early endosomes, a specific sort of intracellular vesicle. The cargos are then transferred to late endosomes, which have different proteins and functions than early endosomes. From endosomes, cargo can go either to lysosomes, where they are degraded, or to the Golgi apparatus, which sends cargo back to the cell surface.

Although many details of clathrin-mediated endocytosis have been uncovered, cell biologists still hotly debate whether early endosomes mature into late endosomes or whether transport vesicles take cargos from early to late endosomes. Unraveling such details will improve our understanding of normal cellular processes and should help in the design of intracellularly



DOI: 10.1371/journal.pbio.0030260.g001

Immunofluorescence showing intracellular compartments containing Rab7 (red), EEA1 (green), and Semliki Forest Virus (blue)

targeted drugs. Andreas Vonderheit and Ari Helenius now provide new insights into this controversy by examining how Semliki forest virus (SFV) is sorted and transported to late endosomes.

Like many animal viruses, SFV enters its host cells using clathrin-mediated endocytosis. One well-established way to study this process is to attach a fluorescent tag to individual virus particles and observe their travels through the cell. Vonderheit and Helenius now track this journey in greater detail than ever before by attaching different colored fluorescent tags to SFV and to protein markers of early and late

endosomes. They then use video-enhanced triple-color microscopy to follow all the markers as they move through living cells. This analysis reveals that the virus is initially present in endosomes containing only proteins associated with early endosomes. Then, Rab7, a late endosome marker that is involved in transport of cargo from early to late endosomes, appears in distinct domains of these early endosomes. Finally, the viral cargo is transferred to a detached organelle that contains Rab7 but no early endosome markers. The researchers show that SFV transport to late endosomes requires Rab7 and the presence of intact microtubules, which often serve as a highway network along which vesicles travel.

The researchers conclude that, at least for SFV, the mechanism underlying sorting and transport from early to late endosomes falls somewhere in between the two existing models for clathrin-mediated endocytosis. Early endosomes, they postulate, have to acquire some characteristics of late endosomes before SFV can be transported to late endosomes in Rab7-positive vesicles. But other cargos, the authors point out, may follow different pathways through the cell.

Vonderheit A, Helenius A (2005) Rab7 associates with early endosomes to mediate sorting and transport of Semliki forest virus to late endosomes. DOI: 10.1371/journal.pbio.0030233

Transcriptional Waves in the Yeast Cell Cycle

DOI: 10.1371/journal.pbio.0030243

Mobilizing an army to march into battle requires the increased activity of hundreds of people, from the quartermaster to the gunnery captain. The stately march of the cell cycle—from the first growth phase, through DNA synthesis, to the second growth phase, and on to mitosis and cell division—also demands increased activity, but of hundreds of genes, from histones to protein kinases. And just as the army must coordinate the shipment of C rations with the movement of its troops, so must the cell coordinate its genetic activities to ensure that raw materials and regulatory molecules are present where and when they are needed. In this issue, Janet Leatherwood, Bruce Futcher, and colleagues describe the waves of gene activity that accompany

the phases of the cell cycle in the yeast *Schizosaccharomyces pombe*.

Using microarrays, the authors examined the expression level of 5,000 genes over the course of the cell cycle. They found that well over 2,000 of these genes undergo slight but observable and statistically meaningful oscillations. Of these, they chose to examine the top 750, an admittedly arbitrary cutoff that nonetheless highlights those whose expression levels rise and fall the most. They identified two broad waves of oscillation, one peaking in early to mid-G2 (the second growth phase) and the other late in G2 at the transition to mitosis. These two peaks were seen even in the 4,000 least cyclic genes, suggesting that many genes may be slightly

upregulated not for adaptive purposes, but simply because some transcription factors inevitably go astray whenever there are lots of them around.

Such broad waves of upregulation are likely due to a simultaneous increase in the activity of multiple clusters of genes, each controlled by separate groups of transcription factors. A variety of cell culture manipulations allowed the researchers to identify eight clusters of genes, the activity of whose members was tightly co-regulated. (In this case, "cluster" refers not to genes physically grouped together on a chromosome, but to genes that are regulated similarly.) Scouring the promoters of these genes confirmed that each cluster was characterized by unique transcription factor binding sites. They also discovered that, as a group, these promoters tended to be longer than average, suggesting they may be more complex than those in non-oscillating genes.

The number of genes within each cluster ranged from only a few to over 100. The largest of them, the Cdc15 cluster, contains genes involved in mitosis, cytokinesis, and formation of

the septum that separates the daughter cells, as well as genes for other functions. Other clusters regulate DNA replication, cell separation, synthesis of the histone proteins that act as spools on which DNA is wound, protein folding and stress response, ribosome biogenesis, and other aspects of the cell cycle.

Two other recent studies in *S. pombe* have found broadly similar patterns, and have identified 407 and 747 genes, respectively, as strong oscillators. There was a heartening degree of overlap, with 171 genes identified by all three studies, and 360 more found in two of the three. Even genes that made the cut in only one study were found likely to oscillate in the other two. Follow-up studies to further explore genes that are coordinated during the cell cycle march should help us to understand how the army of molecules exerts such fine control over both normal and abnormal cell growth and proliferation.

Oliva A, Rosebrock A, Ferrezuelo F, Pyne S, Chen H, et al. (2005) The cell cycle-regulated genes of *Schizosaccharomyces pombe*. DOI: 10.1371/journal.pbio.0030225

of examining complex processes at the level of single cells, while furthering our understanding of how the SOS response is structured in *E. coli*.

Friedman et al. monitored the SOS response by attaching a green fluorescent protein (GFP) to the promoters (the section of DNA responsible for activating a gene) of three SOS genes (*lexA*, *recA*, and *umuDC*). Bacteria expressing these promoter-GFP fusions became fluorescent within minutes of being exposed to UV radiation, visualized using time-lapse fluorescence microscopy. Since GFP fluorescence is directly correlated with the expression of each of the chosen genes (i.e., their promoter activity), the authors could gauge the SOS response rate upon DNA damage.

To induce the SOS response, the authors exposed *E. coli* cells to UV radiation. By monitoring individual cells at two-minute intervals after this dose, Friedman et al. found up to three peaks of promoter activity at roughly 30, 60, and 100 minutes. Although the amount of this activity and the average number of peaks varied between cells, the timing was always similar in different cells, suggesting a highly structured, timed response. When the authors averaged this response over the population, it "washed out" into a single peak—which explains why the three peaks of expression were not previously detected.

A deeper look into the dynamics of the SOS response in single *E. coli* cells showed that it did not correlate with cell size, suggesting the SOS response is not synchronized with the cell cycle. In addition, Friedman et al. repeated their experiments in a bacterial strain lacking the SOS response gene *umuDC*. The peak pattern was altered in this mutant strain, and the precision in the appearance of the peaks was reduced. By re-examining the SOS response in single cells, Friedman et al. have visualized an accurately timed and synchronized DNA repair process. Modulations in response to DNA damage have also been observed recently in individual mammalian cells. Future experiments in *E. coli*—one of the most genetically tractable model systems—should help explain how this timed response is related to the different pathways of DNA repair and shutoff of the response.

Friedman N, Vardi S, Ronen M, Alon U, Stavans J (2005) Precise temporal modulation in the response of the SOS DNA repair network in individual bacteria. DOI: 10.1371/journal.pbio.0030238

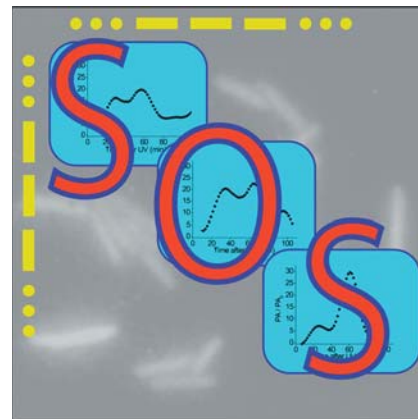
Three New Phases of Repairing DNA Damage in *E. coli*

DOI: 10.1371/journal.pbio.0030239

Any cell that receives a dose of radiation is placed in a dangerous situation. The DNA damage resulting from exposure to such radiation (or any other mutagen) can cause massive rearrangements to genetic information and potentially kill the cell. Bacteria have learned to cope with this threat by activating genes that repair DNA damage and by preventing a cell from dividing before these repairs are completed. In the bacteria *Escherichia coli*, these repair genes form what is known as the SOS response.

The *E. coli* SOS response has been used to study DNA repair for decades, and a great deal is known about how the more than 30 genes involved in the response function. Two proteins figure prominently in this response. The LexA protein acts as a repressor and inhibits the expression of SOS genes under normal conditions; in the event of DNA damage, the protein RecA inactivates the LexA repressor by enhancing its autocleavage into two fragments, which initiates the SOS response. While these initial stages are well understood, how all the SOS genes are coordinated, and ultimately turned off, is only beginning to be explored.

In a new study, Joel Stavans, Uri Alon, and colleagues have closely followed the



DOI: 10.1371/journal.pbio.0030239.g001

Genes involved in the SOS response to DNA damage are expressed in three precisely timed phases

SOS response in individual *E. coli* cells to investigate its dynamics. Previous studies, which monitored the temporal pattern of activation of entire populations of cells, found that SOS genes turned on in one peak upon DNA damage. But Friedman et al. found that SOS genes in individual bacteria respond to DNA damage in three precisely timed phases. This observation reveals the importance

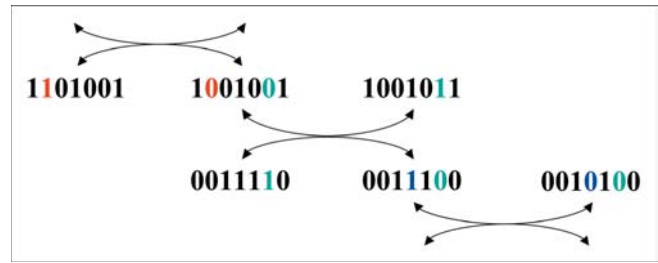
Simple Rules Reproduce a Hub-Shaped Cell Metabolic Network

DOI: 10.1371/journal.pbio.0030261

A map of a cell's metabolic pathways looks like an airline route map on steroids, with hundreds of reactions forming a complex and interconnected network. And just as a few cities serve as destination hubs for many different flights, a few metabolites, such as ATP and NADH, form biochemical hubs within the metabolic network. These molecules are involved in far more reactions than the average, and thereby serve to couple otherwise unrelated reactions within the cell. How did this hub-shaped network arise? In this issue, Thomas Pfeiffer and colleagues employ a computer simulation of a simplified metabolic system to show that two key features in the evolution of a hub system are enzyme specialization and the transfer of chemical groups between metabolites.

The authors created "molecules" from all possible combinations of seven "groups." They began the simulation with seven "enzymes" that catalyze the transfer of one group from one molecule to another. Initially, each enzyme was a generalist—it could take a group from any molecule and donate it to any other. This mirrors one plausible scheme for the actual evolution of cellular biochemistry. Over the course of the simulation, enzymes could mutate to preferentially increase their affinity for one substrate at the expense of others. The number of enzyme types could be increased by "gene duplications." Other parameters allowed the simulated cell to take up and excrete metabolites, and to grow.

As the system evolved, enzymes proliferated and became more specialized, until the final mix included about two dozen enzymes, each of which catalyzed only one or two reactions. As a consequence, some metabolites fell out of use, and the final number of metabolites dropped from 128 to 33. While most took part in only two or three reactions, a few emerged as hubs, participating in eight or more separate reactions. While this mathematical distribution did not match that found in the



DOI: 10.1371/journal.pbio.0030261.g001

For modeling purposes, metabolites can be denoted as binary strings of biochemical groups; enzymes catalyze the transfer of the groups

metabolic network of a whole real cell, it did approximate that of similar-sized sub-cellular metabolic networks. The central importance of group transfer to this structure was brought out when the authors reran the simulation without group transfer. When reactions simply added or removed a group, without transferring it to another molecule, a much simpler network without hubs evolved instead.

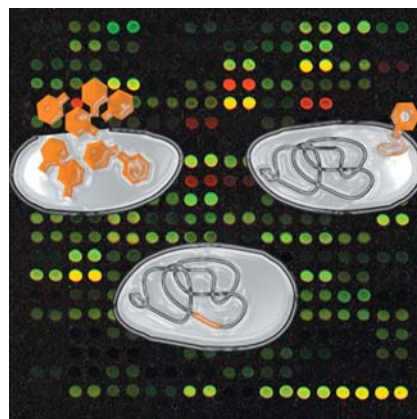
This is not the last word on biochemical evolution, but it does show how an initially generalist metabolism can evolve into the highly specialist system found in all existing cells. Further experiments that model known reactions more closely may be useful in elucidating more details of the evolutionary process that led to the emergence of the biochemical "hub" that characterizes life today.

Pfeiffer T, Soyer OS, Bonhoeffer S (2005) The evolution of connectivity in metabolic networks. DOI: 10.1371/journal.pbio.0030228

Phages Affect Gene Expression and Fitness in *E. coli*

DOI: 10.1371/journal.pbio.0030258

Life is hard for bacteria. Not only must they constantly compete against their comrades for resources and living space, they're also subject to infection by pathogens—viruses called bacteriophages—which can affect their ability to survive and prosper. Two types of bacteriophages threaten bacteria: lytic phages and lysogenic (or temperate) phages. Acquisition of a lytic phage (for example, T2, T4, or T6) is an immediate death sentence for the bacterium; upon infection, a lytic phage subverts the bacterium's biochemical machinery to make copy after copy of itself until the bacterium bursts, or lyses, from the burden. In contrast, a temperate phage (for example, λ phage) can lie dormant for many generations before it co-opts the bacterium's machinery to reproduce, but eventually it, too, lyses the bacterial cell as it releases a host of new phages. From the perspective of the bacterium,



DOI: 10.1371/journal.pbio.0030258.g001

The bacterial virus λ integrates into the *E. coli* genome, where it shuts down the cell's ability to grow on poor carbon sources

it is better to be infected by a temperate phage than a lytic phage because infection with a lytic phage means instant

death, while a temperate phage may lie dormant long enough for the bacterium to reproduce.

Temperate phages achieve dormancy by producing a phage gene product (in the case of λ phage, called *cI*) that represses the production of other phage genes; phage reproduction ceases as long as this repressor is produced. Once infected by a temperate phage, bacteria are protected from secondary infections by various other phages, because the temperate phage prevents the others from becoming established in the cell. But might temperate phage infection confer other advantages on bacterial survival?

Edward Cox's group at Princeton University examined this question by looking for evidence that temperate phage infection triggers changes in bacterial behavior. Working with λ phages, the authors studied how phage

infection affects the regulation of genes that might impact the bacterium's survival by comparing the constellation of genes expressed in uninfected *E. coli* bacteria to those in *E. coli* carrying a dormant λ phage. They found that λ phage caused reduced expression of the bacterial gene *pckA*, which codes for an enzyme that helps bacteria grow on carbon sources (fuels) other than glucose; without functioning *pckA*, bacteria grow normally in an environment containing glucose, but grow only slowly in an environment containing alternative carbon sources such as succinate. *E. coli* carrying λ phage fail to make the *pckA* gene product because the *pckA* gene

is turned off by the virally encoded repressor *cl*. Interestingly, the researchers found evidence that the repressors made by other temperate phages may also be able to turn off *pckA* expression, and that the *pckA* genes of other bacteria related to *E. coli* might also be regulated by temperate phage repressors.

The fact that this relationship between temperate phage repressors and regulation of the *pckA* gene is so well conserved argues that the ability to turn off this gene might be positively selected; therefore, *pckA* repression must confer some sort of survival benefit to the bacterium. It's not clear what this benefit might be, but one explanation is that

slowing bacterial growth in glucose-poor environments might help the bacterium elude detection by the immune system of any animal it invades, increasing its chances of survival. Alternatively, slower bacterial growth might slow down the onset of viral reproduction and eventual lysis. Regardless, it is clear that there is a strong relationship between the temperate phages and the bacteria they colonize. These results have significant implications for the evolution of fitness in these bacterial populations.

Chen Y, Golding I, Sawai S, Guo L, Cox EC (2005) Population fitness and the regulation of *Escherichia coli* genes by bacterial viruses. DOI: 10.1371/journal.pbio.0030229

Are We Underestimating Species Extinction Risk?

DOI: 10.1371/journal.pbio.0030253

Aside from global climate change, loss of biodiversity poses one of the greatest threats to the planet. Last year, the World Conservation Union reported an unprecedented decline in biodiversity, with nearly 16,000 species facing extinction. The biggest threat to the vast majority of these species is loss of habitat. And as habitat loss and degradation proceed nearly unabated, the need to accurately predict the population dynamics and extinction risk of potentially endangered species has never been greater. In a new study, John Drake tests models traditionally used to estimate the likelihood of extinction and shows that because the models ignore a critical parameter in projecting risk, they underestimate extinction rates.

Standard models for predicting extinction assume that population growth and decline are governed by random, or stochastic, variables. The models typically incorporate two major contributors to random variation in population growth rates: changes in environmental conditions and chance fluctuations in population size—caused by variations in individual fitness, random mating behavior, and events that affect just one individual—that are referred to as demographic stochasticity. But since few scientists have tested these models with empirical data, the question remained whether the models were accurately predicting population fluctuations and extinction risk.

To test the reliability of standard stochastic models, Drake used data from experiments with water fleas. He found that the models could accurately predict extinction risk only when there was enough information about variation in individual fitness to account for demographic variability—a finding that undercuts the conventional wisdom that demographic stochasticity is unimportant. Some traditional models do not even include demographic stochasticity.

It's generally assumed that fluctuating environments, a given in the natural world, increase a species's chance of extinction. Drake tested this notion in experiments by manipulating the available food sources in 281 populations of water fleas. The flea populations received either low, medium, or high amounts of food, and Drake kept daily tallies of population number and extinctions. When he tried to predict the extinctions using traditional models, he couldn't.



DOI: 10.1371/journal.pbio.0030253.g001

Experiments with *Daphnia magna*, the water flea, show that traditional extinction models may be underestimating extinction risk

To account for the discrepancy between model and data, Drake began to investigate a possibility raised by recent theoretical research that population density and individual interdependence might affect a major component of the model—demographic stochasticity. The idea is that if organisms interact in their environments—which of course they do—then these interactions will likely affect an individual's probability of dying or reproducing, which ultimately affects species survival. Drake calls this variable density-dependent demographic stochasticity.

Drake used half of the experimental data generated from testing the effects of environmental variability on water flea survival to select his models and estimate the range of parameters that might affect extinction, and the other half to test the models' reliability. From the estimated parameters, Drake wrote a computer program to simulate all the possible population outcomes and predict extinction rates. One set of

simulations included a parameter for density-dependent random interactions and another did not.

When Drake analyzed all the possible outcomes, it turned out that manipulating food supply didn't have as great an effect on extinctions as predicted—possibly because individual water fleas live too long compared to the frequency of the environmental fluctuations. Only when density-dependence was included did the models match the observed extinction rates in the flea experiments. When density dependence was not included, extinction rates were greatly underestimated.

Drake's results underscore the importance of bolstering extinction models with empirical validation—and of accounting

for population density—to accurately evaluate risk and enhance recovery programs for at-risk populations. As threats to endangered species continue to mount, biologists will need ever more robust methods to estimate extinction risk. Unfortunately, field biologists typically can't generate the large, high-quality datasets that led to the precise predictions reported here. Conservation efforts will depend on developing methods of generating reliable predictions with the limited data available from the field.

Drake JM (2005) Density-dependent demographic variation determines extinction rate of experimental populations. DOI: 10.1371/journal.pbio.0030222

Patterns of Genetic Variation Reveal Plant's Evolutionary Roots

DOI: 10.1371/journal.pbio.0030231

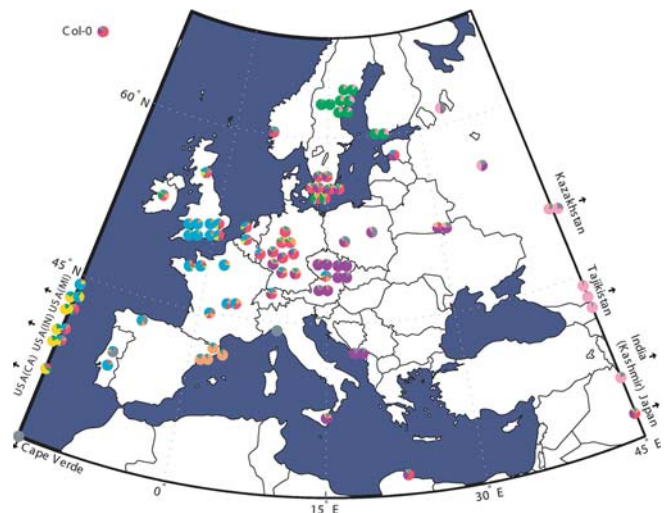
Biologists have developed ever more sophisticated ways to find molecular traces of natural selection. These traces—which occur as variations in DNA sequence, or polymorphisms, between and within species—are thought to harbor the genetic basis of adaptive events.

The study of natural selection at the molecular level has long been dominated by Kimura's theory of neutral evolution, which argues that most polymorphisms (in both DNA and protein sequence) have minor or no selective effect, and are governed by random, not selective, processes. The strength of this theory is that it leads to clear predictions that can be tested to identify those polymorphisms that really are subject to selection. Even though there's a large body of literature devoted to the statistical testing of selective neutrality, these tests are generally based on theoretical models, the assumptions of which have largely been untested. Only recently have the necessary quantities of data for testing the merits of these models become available, thanks to high-throughput genotyping and sequencing technologies.

Working with *Arabidopsis thaliana*, the first plant genome sequenced, Magnus Nordborg, Joy Bergelson, and their colleagues investigate a global survey of polymorphism patterns in the genomes of 96 plants. The scale of their study affords robust insights into the genomic pattern of polymorphism of the plant and sheds light on its demographic history. The results also lay the foundation for future work on the genetic basis of *A. thaliana* variation while challenging the assumptions of standard mathematical models for determining whether a gene is under natural selection in the plant.

Nordborg and colleagues sequenced 876 short genome fragments of 96 *A. thaliana* plants from both worldwide natural populations and laboratory stocks. In total, they described 44,000,000 DNA bases of genetic material, which revealed 17,000 polymorphisms, either in the form of single changes in DNA sequence (single nucleotide polymorphisms, or SNPs) or as losses or additions of DNA sequence between individual plants.

The level of polymorphism in *A. thaliana* is unexpectedly high for a plant that is highly self-fertilizing. To see if these polymorphisms were uniformly shared across plant populations, or had a distinct structure, Nordborg and colleagues grouped a subset of the *A. thaliana* plants into populations based on their geographic origin. Although they found that individuals within a population harbor much of the variation that is typical of the species worldwide, it appeared that some of the variation was specific to particular geographic regions. Furthermore, closely



DOI: 10.1371/journal.pbio.0030231.g001

The global geographic structure of variation in *Arabidopsis* is shown by the clusters of similar pie charts signifying patterns of isolation (each chart represents an *Arabidopsis* accession)

related plants were almost always from the same local region. The authors suggest that is what would be expected for a sexually reproducing species found worldwide.

With such a large data set, it also becomes possible to see if the underlying assumptions of mathematical models commonly used for determining whether a gene is under selection are appropriate for *A. thaliana*. Nordborg and colleagues found that the patterns they observe do not fit the standard neutral model of evolution, which is expected to explain most genetic variation. This model is the benchmark against which researchers pinpoint the signature of selection at particular genes. The authors caution that "commonly used 'tests of selection' are simply not valid in *A. thaliana*."

Nordborg and colleagues have provided a wealth of detail to our understanding of genetic variation in *Arabidopsis* on a genome-wide scale. Future research can now begin to use this *Arabidopsis* genetic footprint to find the exact variations that contribute to useful plant traits—and plumb its genome for evolutionary clues.

Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, et al. (2005) The pattern of polymorphism in *Arabidopsis thaliana*. DOI: 10.1371/journal.pbio.0030196

For Long-Lived Flies, It's Calorie Quality, Not Quantity, That Matters

DOI: 10.1371/journal.pbio.0030237

In April, the United States Centers for Disease Control and Prevention released a study challenging the conventional wisdom that eating less promotes longevity. The study found that the very thin run roughly the same risk of early death as the overweight. And now the tide seems to be turning against a common explanation for the long-standing observation that restricting food in lab organisms from yeast to mice prolongs life.

Many studies have indicated that it's calorie reduction, rather than the specific source of calories, that increases longevity. That this effect occurs in such diverse organisms suggests a common mechanism may be at work, though none has been definitively characterized. And while calorie restriction enhances longevity in mice, it has not always done so in rats. In a new study, William Mair, Matthew Piper, and Linda Partridge show that flies can live longer without reducing calories but by eating proportionally less yeast, supporting the notion that calorie-restriction-induced longevity may not be as universal as once thought.

Dietary restriction in *Drosophila* involves diluting the nutrients in the fly's standard lab diet of yeast and sugar to a level known to maximize life span. Since both yeast (which contributes protein and fat) and sugar (carbohydrates) provide the same calories per gram, the authors could adjust nutrient composition without affecting the calorie count, allowing them to separate the effects of calories and nutrients. The standard restricted diet had equivalent amounts of yeast and sugar (65 grams each) and an estimated caloric content of 521, while the yeast-restricted (65 g



DOI: 10.1371/journal.pbio.0030237.g001

Contrary to popular belief, life span extension by dietary restriction in *Drosophila* is not explained by calories

yeast/150 g sugar) and sugar-restricted (65 g sugar/150 g yeast) diets each had just over 860 calories. The control diet for the flies had equivalent amounts of sugar and yeast (150 grams), amounting to an estimated 1,203 calories.

First, the authors had to make sure the flies didn't change their eating behavior to make up for a less nutritious diet. (They didn't.) Reducing both nutrients increased the flies' life spans, but yeast had a much greater effect: reducing yeast from control to dietary restriction levels increased median life span by over 60%.

In a previous study, Mair et al. showed that flies that were switched from dietary-restricted diets to control diets soon began to die at the same rates as flies accustomed to the control diet. In

this study, the authors studied the effects of switching yeast and sugar. Forty-eight hours after being switched from normal diets to yeast-restricted diets, flies were no more likely to die than flies fed the yeast-restricted diet from the beginning. In contrast, those switched from the standard restriction diet to the sugar-restricted diet began to die at the same rate as flies on the control diet.

The authors also ruled out the possibility that bacteria—attracted to high-nutrient food—might be influencing fly survival. Altogether these results make a strong case that calories per se are not the salient factor in prolonging life—at least in fruitflies. The dramatic impact of reducing yeast suggests that protein or fat plays a greater role in fly longevity than sugar. This in turn suggests, the authors argue, that yeast and sugar trigger different metabolic pathways with different effects on life span.

Why might different factors promote longevity in flies and rats? It could be that the calorie-restriction/longevity paradigm needs more rigorous review—though a vast body of literature does support it. Or it may be that the animals use the same strategy for dealing with food shortages—shifting resources from reproduction to survival, for example—but have evolved different mechanisms for doing so that reflect each species's life history, diet, and environment. Whatever explains the disparity, this study should give researchers interested in caloric restriction plenty to chew on.

Mair W, Piper MDW, Partridge L (2005) Calories do not explain extension of life span by dietary restriction in *Drosophila*. DOI: 10.1371/journal.pbio.0030223

Host–Parasite Battles Shed Light on the Evolution of Gene Expression

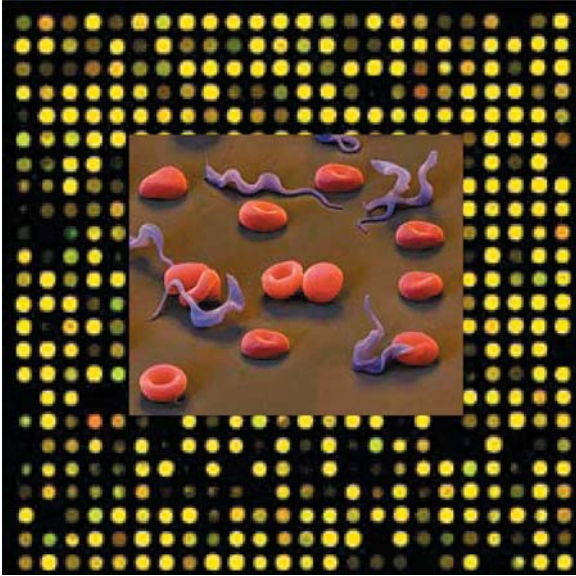
DOI: 10.1371/journal.pbio.0030252

In human–pathogen encounters, the battle for advantage plays out at the level of gene expression. Hosts will stand a better chance of surviving if their genes confer resistance to diverse pathogens, while pathogens need genes that promote virulence and infection. To understand this game of evolutionary one-upmanship, biologists study the genetic basis of resistance and infection by investigating how changes in an organism's genetic makeup, or genotype, affect its physiological and physical makeup, or phenotype.

In a new study, Scott Nuismer and Sarah Otto detail how host–parasite interactions shape the changes in gene expression that alter an organism's ability to induce or resist infection. They

find that gene expression for host and parasite follows quite different evolutionary paths: hosts express as many different gene variants, or alleles, as possible, while parasites express very few alleles. It's in the host's interest to have as many genetic weapons as possible that can recognize a foreign invader, while it's in the parasite's interest to reduce the number of recognizable molecules for a host to latch on to and destroy. Even though these results are intuitive, this phenomenon has not been shown before.

To model the evolution of gene expression levels in a host–parasite interaction, Nuismer and Otto started with a single gene A in hosts and a single gene B in parasites with two alleles each



DOI: 10.1371/journal.pbio.0030252.g001

Interactions between hosts and parasites shape changes in gene expression, potentially maximizing parasite recognition for hosts while minimizing detection for parasites (such as red blood cells and trypanosomes)

(*A* or *a*, and *B* or *b*) that are involved respectively in resistance in the host and promoting infection by the parasite. Nuismer and Otto's model allows gene expression levels to be regulated by an additional modifier gene in hosts and parasites. The two variants of the modifier gene (*M* and *m*) alter the expression of the host

gene *A* or parasite gene *B*. As a result of host–parasite interactions, the alleles at the modifier gene either evolve to increase expression of only one allele or to co-express both alleles.

When parasite and host are allowed to interact, the model shows that host resistance alleles typically evolve toward co-expression while parasite infection alleles evolve toward single expression. By expressing more than one gene at a time, the host can recognize a greater diversity of parasites. But what's good for the host is bad for the parasite. Hosts benefit from a wider array of parasite recognition systems, while parasites benefit from expressing a narrow range of antigens to evade the host recognition system.

Human immune cells, for example, can recognize billions of different antigens, which then triggers an immune response against the foreign substance and increases the chance of surviving the infection. Parasites, however, generally express only one of many possible antigen alleles. The parasite responsible for African sleeping sickness expresses only one of thousands of surface receptor genes, which offers the host fewer opportunities for detection.

Nuismer and Otto's model provides a framework for understanding empirical observations of allele expression in known host–parasite interactions and may well help explain similar modifications in allele expression in other systems. Because the model also provides testable predictions, it should be useful in interpreting data from a wider range of species and interactions, furthering our understanding of the evolutionary forces that shape infection and resistance and ultimately influence how genes evolve.

Nuismer SL, Otto SP (2005) Host–parasite interactions and the evolution of gene expression. DOI: 10.1371/journal.pbio.0030203

Cutting through the Clutter: How the Brain Learns to See

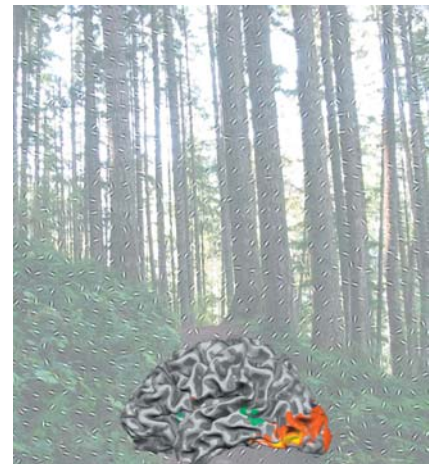
DOI: 10.1371/journal.pbio.0030256

Most of us don't have much trouble recognizing what we see. Whether it is a face in a crowd, a bird in a tree, or papers on a desk, our brains expertly distinguish the target from the clutter. It is a simple skill most of us take for granted, but object recognition is not hard-wired. As we navigate our environment, the brain's visual centers continually reorganize themselves, classify novel features, and learn to pick out important objects from the background. Just how the human brain does this is not well understood, but new research by Zoe Kourtzi and colleagues may have uncovered some important clues.

To investigate how the human brain learns to separate targets (signal) from noise Kourtzi et al. showed subjects pictures of novel shapes embedded in a cluttered background and asked the subjects to determine whether or not the shapes were symmetrical. The researchers recorded the subjects' responses while using functional magnetic resonance imaging (fMRI)

to measure neuronal activity in brain regions associated with visual processing. Each subject was tested using two sets of novel shapes: high-salience shapes (shapes easily distinguished from the background), and low-salience shapes (shapes camouflaged by the background). After the initial testing, the subjects were trained to recognize a subset of the new shapes from each group, and then re-tested.

Visual input is thought to go through a hierarchy of processing centers that transform retinal images into complex objects and scenes. Kourtzi et al. recorded responses from both early (V1, V2, Vp, and V4) and late (lateral occipital cortex) stages of visual analysis in 26 subjects. The authors found that subjects demonstrated an increased number of correct responses for shapes they encountered during the training sessions, regardless of the type of background the shapes were presented on. By contrast, the fMRI responses differed dramatically, depending on whether the surroundings made the shapes easy or



DOI: 10.1371/journal.pbio.0030256.g001

The human brain learns to detect the contours of target objects in cluttered scenes by recruiting early and higher centers of visual analysis

difficult to detect. Low-salience shapes triggered an increased fMRI response across all brain regions following training;

high-salience shapes precipitated a decrease in fMRI response in the regions of the lateral occipital cortex, but produced no change in any of the early visual areas (V1, V2, Vp, and V4).

These results demonstrate that the ability to learn to detect novel shapes is independent of the degree of difficulty, but suggest that the brain employs different mechanisms of perceptual learning depending on whether the objects stand out from their surroundings, or are obscured by them. Learning to detect highly camouflaged shapes results in increased brain activity levels that are presumed to reflect an increase in signal processing at the level of both the early

visual areas and higher levels of cortical analysis. On the other hand, the reduction of neural activity that occurs during learning of more distinctive shapes likely reflects efficient neural coding of the critical features for their recognition at later stages of visual analysis.

According to Kourtzi and her colleagues, their results provide evidence that the visual brain is capable of tailoring the mechanism of perception to best suit the task. When the signal is weak—as in the case of viewing camouflaged targets—learning amplifies neural responses to the target shapes and drowns out the noise. But when the signal is strong—as in the case of viewing easily distinguishable,

highly salient targets—neural activity in the visual cortex is reduced, possibly because training engages smaller populations of neurons that respond much more selectively to distinctive features of the stimulus.

In other words, all visual stimuli are not treated equally, and with just cause: the brain's unique ability to treat ambiguous signals differently than robust ones likely allows it to optimize neural coding, and in doing so, learn to increase detection of a broad spectrum of visual signals.

Kourtzi Z, Betts LR, Sarkheil P, Welchman AE (2005) Distributed neural plasticity for shape learning in the human visual cortex. DOI: 10.1371/journal.pbio.0030204

Attraction to Motion

DOI: 10.1371/journal.pbio.0030251

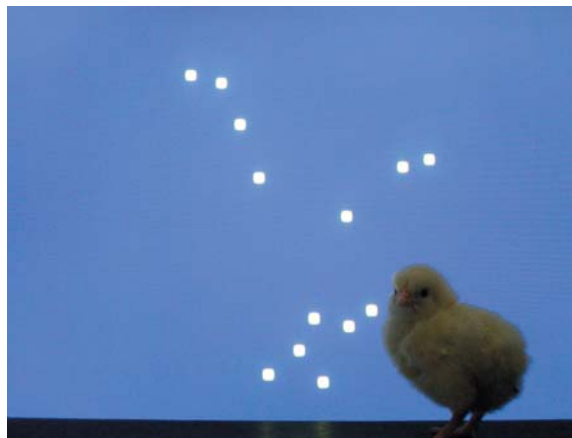
Anyone with a passing familiarity with animal behavior knows the classic photo of Konrad Lorenz trailed by a gaggle of goslings. Lorenz showed that geese hatched in an incubator identified with the first moving stimulus they saw within 36 hours after birth. When the first thing was Lorenz—or more accurately, his boots—the baby geese imprinted on him.

But what can a newborn recognize? What elements in the visual world are their brains preprogrammed to process? In a new study, Giorgio Vallortigara, Lucia Regolin, and Fabio Marconato take advantage of the natural imprinting behavior of newly hatched chickens to study motion perception. It's clear that animals are more likely to respond to something that moves than to something that doesn't and that the motion of animate objects (biological motion) has uniquely identifiable characteristics. Is this identification innate or is it something an animal learns through experience?

To tackle these questions, the researchers presented chicks with artificially induced motion patterns. The chicks were immediately drawn to and stayed close to iconic patterns of animal motion, also known as biological motion. Interestingly, the chicks didn't distinguish between friend and foe: they were just as likely to approach a cat as a hen.

To test the chicks' preference for biological motion, the researchers took advantage of the fact that many vertebrates—whether they be geese, chickens, or humans—move in a distinctive, coordinated manner. By strategically positioning tiny lights at key points along an animal's torso and limbs, it's possible to strip a moving object of all extraneous traits like shape, texture, and color. People watching such “point light display” animations can easily recognize a person walking, discern the person's gender, and even identify a friend.

Animals are similarly endowed with the ability to extract information from point light displays. To test the chicks' preference for different types of motion, Vallortigara et al.



DOI: 10.1371/journal.pbio.0030251.g001

Are you my mom? “Point light animations” test chicks' preference for biological motion

created four animations from 13 points of light. One represented a walking hen, a second created the impression of a “rotating rigid hen-like object” (true animal movement is fluid, though constrained by the skeleton), a third moved in arbitrary directions, and a fourth, the “scrambled hen,” conveyed biological motion, but of an unknown creature (as perceived by human observers). Chicks were hatched in darkness to make sure that the first thing they saw was one of the animations.

The chicks consistently approached the walking and scrambled hen, showing far less affinity for the rigid and random motion, suggesting a predisposition toward the

movement typical of vertebrates. As a control, the authors generated an animation of a walking cat. Sure enough, chicks approached the walking cat as often as they approached the walking hen. Luckily for chicks, encounters with cats are not normally likely to precede encounters with a mother hen.

These results suggest that chicks have evolved a predisposition to notice objects that move like vertebrates, which may maximize the probability of imprinting on the object most likely to provide food and protection after birth. This predisposition likely guides the learning that occurs during the imprinting process, when the chick learns how to distinguish mom from other hens, and hens from cats. Since both birds and mammals (tested in four-month-old human infants) show a preference for biological motion, the authors conclude, these results suggest that this preference is hard-wired into the vertebrate brain. With this new model, researchers can investigate the motion-specific features that guide the chicks' behavior and how the brain processes biological motion.

Vallortigara G, Regolin L, Marconato F (2005) Visually inexperienced chicks exhibit spontaneous preference for biological motion patterns. DOI: 10.1371/journal.pbio.0030208