Why Bad Boys Always Get the Girl and Other Tales of Evolutionary Madness

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Biologists studying the evolution of mate choice have their work cut out for them. Not only is there little agreement on how best to determine the interplay between mate choice and fitness, there's not even consensus on how to estimate fitness. (Fitness being an individual's success in passing their genes on to future generations.) Why females fall for captivating males that give them nothing but trouble is especially puzzling. Such males tend to contribute their genes and little else, leaving the female to spend precious resources bearing and raising her young, efforts that often cut her life short. Of course, females are not all innocence and light in the mating game: some black widow species, for example, famously make dad a post-coitus snack. Still, females typically incur more costs than males in rearing offspring, especially when they choose flashy mates. So why do they do it?

One model holds that females put up with deadbeat dads because the benefits, though indirect, outweigh the costs: that is, attractive males are more likely to grace their offspring with good genes that increase survival ("I'm so fit I can afford to waste energy on this excessively exuberant tail") or with sexy traits that confer mating success ("my magnificent plumage may decrease my survival, but I get lots of dates"). Another model argues that selection for such indirect benefits is much weaker than direct selection on genes that affect mate preference and thus is likely to exert little influence on mating preference.

In a new study, Megan Head and her colleagues navigate this intellectual minefield by studying the mating behavior of crickets. The authors paired females with either "attractive" or "unattractive" males (see below) and measured a variety of fitness components to estimate the overall fitness consequences of the various unions. Female crickets, they found, pay a high price for mating with attractive males. But when the fitness consequences for their sons and daughters are taken into account, mate costs are balanced by, and may even be outweighed by, the indirect benefits of spawning offspring with elevated fitness. This benefit stems in large part, the authors argue, from siring sexy sons.

How does one distinguish lothario from loser in the cricket world? By running a cricket tournament, of course. For crickets to mate successfully, the female must mount the male so their genitalia align. Noting that females produce more eggs for males they mount quickly, the authors use time to mount as a measure of male attractiveness.

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An attractive cricket?

In the first round of the tournament, Head and colleagues paired males with a randomly assigned female; after mounting, but before copulation, the couples were separated. This continued until half of all females had mounted a male. (Under tournament rules, crickets had to be in the first half of a given category to qualify for the next round.) In round two, a new female was randomly assigned to each male. Males that had been mounted in the first round and remounted in the second were deemed "attractive." Males rebuffed in the first round that remained unmounted longest in round two were "unattractive." Females were randomly assigned to males that were either attractive or unattractive. Equivalent males were swapped out every seven days to control for any individual quirks that might bias the results.

To estimate the total fitness of the participants, the authors measured both direct and indirect fitness components, such as female hatching success and reproductive effort (egg number and size), as well as sons' attractiveness and the number of eggs laid by daughters. Females that mated with attractive males produced daughters that laid more eggs within a given time and sons that were more attractive, though they had lower survival. Thus, by evaluating both the direct effects of female lifetime fecundity and the indirect effects of offspring fitness, the authors determined the net consequences of a mating strategy. And once again, it's mom's sacrifices that keep things on track.

With this approach, Head and colleagues bridge the gap between empirical studies of mating choice evolution, which rely largely on rate-insensitive measures (such as counting grandchildren), and theoretical studies, which typically use rate-sensitive measures. Their results suggest that there may be selection for choosing costly mates and that generating a reliable analysis of the fitness consequences requires a long view: look at the reproductive success of mom's sons and daughters before judging her bad taste in mates.

Head ML, Hunt J, Jennions MD, Brooks R (2005) The indirect benefits of mating with attractive males outweigh the direct costs. DOI: 10.1371/journal.pbio.0030033

Novel Enzyme Shows Potential as an Anti-HIV Target

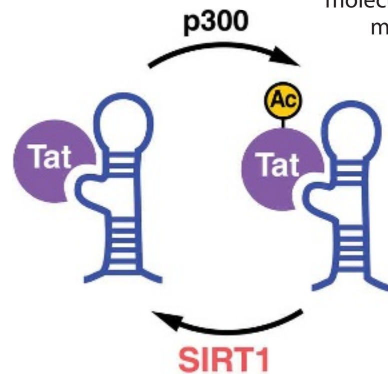
DOI: 10.1371/journal.pbio.0030074

At just 9.8 kilobases, the HIV genome pales in comparison to the 3.2 gigabases of its human and nonhuman primate targets. The compact retrovirus encodes just 14 proteins, which play different roles in promoting viral infection and virulence. As a retrovirus, HIV uses the host's cellular machinery—including RNA polymerases, which carry out transcription—to copy its RNA genome into DNA and infiltrate human chromosomal DNA. Once the virus is integrated (now called a provirus), its genes can be transcribed.

Adept as HIV is in exploiting its host's molecular resources, the virus can't establish a foothold without the services of its skeleton crew. The HIV transcription factor Tat ("transactivator of transcription"), for example, is an essential regulator of HIV gene expression. Without Tat, HIV transcripts don't reach full length and can't effect viral replication. In a new study, Melanie Ott and colleagues identify an enzyme that regulates viral transcription by modifying Tat.

The regulation of HIV genes depends on a complex interplay between proviral DNA, cellular proteins and transcription factors, and Tat. Unlike most transcription factors, Tat activates transcription by binding to RNA, specifically to a bulging "stem-loop" structure that forms at one end of all viral transcripts called the *trans*-acting responsive element (TAR). Tat binding to TAR requires recruiting the enzyme cyclin-dependent kinase 9 (CDK-9) to the HIV promoter (where transcription begins). CDK-9 chemically modifies the RNA polymerase and enhances its transcribing efficiency.

The transcription process—including the labyrinthine protein–protein and protein–DNA (and in the case of Tat, protein–RNA) interactions—is highly regulated. One process that figures prominently in this regulation is acetylation, which adds an acetyl group (a molecule made of oxygen, hydrogen, and carbon) to a molecule or protein. Histone acetylation was long thought to influence transcription by regulating the structure and function of chromatin, which is an assembly of proteins (mostly histones) and DNA. Another, more widely accepted model proposes that histone acetylation controls transcription by recruiting cofactors required for transcription.



DOI: 10.1371/journal.pbio.0030074.g001

Recycling of Tat through deacetylation by SIRT1

Acetylation and deacetylation enzymes can also target other proteins. Of the three classes of deacetylases known to modify human histones, the sirtuins (SIRT1–7) appear to preferentially target a number of nonhistone proteins. Ott and colleagues first tested the ability of all seven SIRT proteins to deacetylate Tat by placing them in a test tube with Tat proteins. Though three SIRT enzymes caused Tat acetylation, only one, SIRT1, is a nuclear enzyme, like Tat, suggesting that SIRT1 might work similarly in living cells.

Ott and colleagues went on to show that transcription via Tat occurs in the presence of SIRT1, but not when SIRT1's catalytic center is removed. Experiments using cells taken from transgenic mice lacking SIRT1 demonstrated that introducing human SIRT1 enzymes increased Tat's transcriptional effects in a dose-dependent manner, while treating cells with the small molecule HR73, a derivative of a molecule that inhibits the yeast version of the SIRT1 protein, caused a 5-fold reduction in HIV transcription.

The authors propose a cycle of transcriptional transactivation in which SIRT1 deacetylates Tat at the HIV promoter. Deacetylated Tat associates with CyclinT1 and TAR, and leads to transcription. Tat acetylation dissociates Tat from CyclinT1 and TAR, and transfers Tat to the elongating polymerase complex. Since acetylated Tat can't recruit CyclinT1 and CDK-9, the authors explain, a new round of transcription requires that new, unacetylated Tats are produced or existing Tats are deacetylated. Thus, efficient viral replication depends on adequate Tat supplies. And since HIV gene expression relies on SIRT1's enzymatic activity, inhibiting SIRT1 could prove to be a promising anti-HIV therapy. Future study will have to verify whether inhibiting SIRT1 can successfully put the brakes on HIV transcription and control the virus (See also "A New Paradigm in Eukaryotic Biology: HIV Tat and the Control of Transcriptional Elongation" [DOI: 10.1371/journal.pbio.0030076]).

Pagans S, Pedal A, North BJ, Kaehlcke K, Marshall BL, et al. (2005) SIRT1 regulates HIV transcription via tat deacetylation. DOI: 10.1371/journal.pbio.0030041

Hominids Lose Control

DOI: 10.1371/journal.pbio.0030073

What makes us human? From a philosophical perspective, the answer may lie in part in our apparently unique need—and self-awareness—to ask the question in the first place. From a biological perspective, the answer lies in part in the sequence of our DNA. While fossil evidence has provided a rough draft of the story of human evolution, much more remains to be learned about the path our genes followed, a path that diverged millions of years ago from our closest living hominid relatives, the chimp and bonobo. Charting differences between human genomes and those of our evolutionary relatives—both near and distant—has become a powerful tool for filling in the gaps in the human fossil record.

Comparing the human genome to the genomes of other great apes can provide a window into the molecular changes that may ultimately spell the difference between human and nonhuman primates. That task was recently aided by the release of the draft sequence of the chimpanzee genome. Comparing the protein-coding sequences of human and chimp has identified molecular dissimilarities between us, which is to be expected. Though many differences between species can be explained at the molecular level by differences in protein structure, where and when a given protein is produced can be just as or even more important. Differences in protein expression arise from sequences in non-coding DNA that influence the timing and regulation of protein production and action.

In a new study, Peter Keightley and colleagues conduct parallel comparative genomics studies—comparing regulatory regions in the chimp and human genome with those of mouse and rat—and make a startling discovery. The hominid lineages show a surprising lack of selective constraint—deleterious mutations have apparently accumulated—compared to the rodents, racking up an estimated additional 140,000 harmful mutations fixed, or retained, in the human and chimp lineages since they diverged. Such mutations have been selectively eliminated in mouse and rat.

The authors focused on DNA sequences making up the bulk of

gene-regulating elements—regions immediately preceding or following protein-coding sequences, as well as the first intron of each gene (an intron is a non-coding DNA sequence squeezed between two adjacent coding fragments). The degree of conservation in these areas was weighed against the conservation in other nearby non-coding sequences, which were assumed to be free of selective constraints. Keightley and colleagues found marked conservation in the regulatory regions between mice and rats, but nearly none between humans and chimps. This result suggests that the gene-regulating elements of hominids are subject to nearly unfettered mutation accumulation, likely due to an absence of natural selection forces strong enough to stabilize the ancestral sequences common to both human and chimpanzee.

How can one explain these puzzling results? Keightley and colleagues propose that selection is ineffective against mildly unfavorable mutations in the gene-regulating regions because of the small effective population size in the evolutionary history of hominids.

What do these results suggest for the future of human evolution? It's unlikely that the regulatory gatekeepers of our genome will allow mutations to spin out of control. Even if the number of unwanted mutations were to increase, stronger natural selection against them is likely to develop in parallel, Keightley and colleagues explain, protecting our fitness from a downward spiral. The authors' results support the notion that population size exerts a powerful influence on evolutionary changes at the molecular level and that many changes in gene control regions are under weak selection. With each new sequenced genome added to the comparative genomics lexicon, scientists are becoming increasingly conversant in the grammar and syntax of gene sequences—and filling in more and more gaps in the human story, letter by letter.

Keightley PD, Lercher MJ, Eyre-Walker A (2005) Evidence for widespread degradation of gene control regions in hominid genomes. DOI: 10.1371/journal.pbio.0030042

An HIV Protein Plays a Surprising Role in Gene Activation

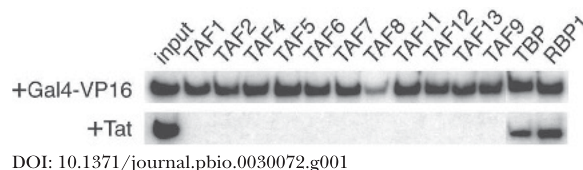
DOI: 10.1371/journal.pbio.0030072

Retroviruses are expert manipulators when it comes to co-opting their host's cellular resources. A great deal of human complexity stems from the vast repertoire of proteins and mechanisms dedicated to the business of regulating gene expression, and retroviruses like HIV have evolved myriad ways of redirecting that machinery to their own benefit.

Humans and other eukaryotes have three types of RNA polymerases, each charged with transcribing different types of genetic elements. RNA polymerase II transcribes protein-coding genes. RNA polymerases join with so-called general transcription factors to form a pre-initiation complex (PIC) on the gene's promoter, where it binds to region rich in thymine (T) and adenine (A) named the TATA box. The first transcription factor to associate with the TATA box is called TFIID, a large protein complex containing a protein that binds the TATA box (aptly named the TATA-box-binding protein, or TBP) and several cofactors called TBP-associated factors (TAFs). PIC assembly sometimes also requires activator proteins, which can enhance transcriptional activity by supporting proper elongation of nascent transcripts.

Tat, an activator encoded in the HIV genome, is required for HIV gene activation and viral replication. It affects these processes, the current model holds, by stimulating transcript elongation and increasing RNA polymerase's processing efficiency. In a new study, Tamal Raha, Grace Cheng, and Michael Green work with human cell lines and find evidence that Tat can also stimulate PIC assembly.

While most transcription factors bind to DNA, Tat binds to an area at the end of newly emerging viral RNA called the transactivation response element (TAR). Once bound, Tat recruits a cellular complex called P-TEFb (consisting of two subunits) to the HIV promoter, and enhances RNA polymerase's transcribing capacity. Previous studies in yeast had shown that activators appear to stimulate transcription complex assembly, leading the authors to ask whether Tat could play a similar role.



DOI: 10.1371/journal.pbio.0030072.g001

An unexpected mechanism of HIV-1 Tat action

To study this question in living human cells, Green and colleagues turned to chromatin immunoprecipitation, a technique that detects proteins bound (directly or indirectly) to DNA. Working with three well-known effectors of transcription—an activator (Gal4-VP16), a transcriptional enhancer, and another viral activator called E1a—the authors show that what's true for yeast also holds for mammals, or at least for the human cell lines investigated here. Each effector was required for PIC assembly, which was in turn required to activate transcription.

The big surprise came in the next round of experiments, which explored Tat's influence on transcription and PIC assembly on the HIV promoter. As expected, transcription factors were "virtually undetectable" at the core promoter in the absence of Tat. Adding Tat recruited all the usual transcription factors to the promoter and increased transcription. But none of the TAFs that normally associate with TFIID were found. When the authors used the activator Gal4-VP16 to initiate HIV transcription, every one of the 11 TAFs studied appeared. None of them did so in the presence of Tat, suggesting that Tat-mediated HIV transcription doesn't rely on TAFs. Green and colleagues confirmed this hypothesis in experiments showing that Tat-driven transcription proceeded as usual in cells lacking TAFs. And they demonstrated that it is Tat—along with its cofactor P-TEFb, which is normally bound to RNA through Tat—that recruits the TAF-deficient TBP.

Altogether, these results show a surprising new role for Tat in stimulating assembly of a transcription complex. What's more, the complex lacks the TAFs typically linked to TBP in mammalian cells. Because their experiments analyzed only transcription complex assembly, the authors are careful to note that Tat may well stimulate assembly in addition to promoting transcription elongation. And it may be this resourcefulness that makes Tat such a potent activator—and HIV so hard to control. (For more on Tat's role in HIV transcription, see "Novel Enzyme Shows Potential as an Anti-HIV Target" [DOI: 10.1371/journal.pbio.0030074] and "A New Paradigm in Eukaryotic Biology: HIV Tat and the Control of Transcriptional Elongation" [DOI: 10.1371/journal.pbio.0030076].)

Raha T, Cheng SWG, Green MR (2005) HIV-1 tat stimulates transcription complex assembly through recruitment of TBP in the absence of TAFs. DOI: 10.1371/journal.pbio.0030044

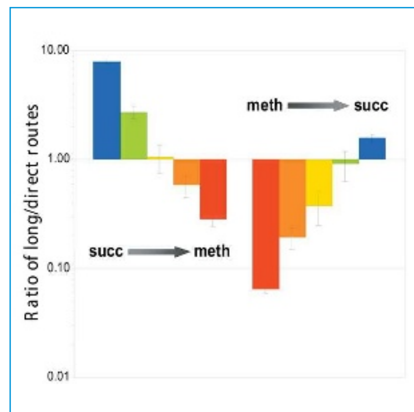
How Bacteria Stopped Worrying and Learned to Love…Formaldehyde

DOI: 10.1371/journal.pbio.0030055

Poring over the vast amount of sequence and genetic information now available for many organisms, scientists frequently encounter what appear to be redundant biochemical pathways. Redundant pathways take the same starting material and transform it into the same product, but through different routes. Why should cells maintain redundant pathways?

An interesting case is that of *Methylobacterium extorquens*, a bacterium that can grow on organic molecules with a single carbon atom such as the alcohol methanol. Bacteria in this species first oxidize methanol into formaldehyde, then use formaldehyde to make serine—the entry point for the synthesis of many of the cell's building blocks—via two apparently redundant pathways. The short pathway is a direct (non-enzymatic) reaction of formaldehyde with tetrahydrofolate to make methylene-tetrahydrofolate, which donates a single carbon atom for serine synthesis. A hypothesized long pathway could also lead to methylene-tetrahydrofolate through a long series of enzymatic reactions, one of which consumes energy.

Christopher Marx and colleagues now demonstrate that the bacteria modulate their use of each pathway during the course of acclimation to growth on methanol. In the process the authors offer new insights into the bacteria's rapid disposal of formaldehyde, a toxic chemical that would pickle cells in a minute if it was allowed to accumulate.



DOI: 10.1371/journal.pbio.0030055.g001

The short and long (pathways) of formaldehyde metabolism

The authors noticed that the short pathway transferred both hydrogen atoms of formaldehyde to the serine molecule while the long pathway transferred only one. If they fed bacteria methanol in which hydrogen had been replaced with its heavier form, deuterium: the resulting serine was slightly heavier than normal, as a result of acquiring one—or two—deuterium atoms.

By measuring the ratio of the two serine forms, Marx and colleagues inferred the relative contribution of the two pathways to serine synthesis. The long pathway dominated, accounting for eight times more serine than the short pathway, when cells first encountered methanol. But the situation was reversed after the cells acclimated to methanol: then the short pathway produced 15 times more serine than the long pathway.

The authors also measured absolute amounts of formaldehyde processed by each pathway, using methanol marked with a heavy form of carbon (^{14}C). Although the relative contribution of the long pathway decreased during ramping-up to methanol growth, the absolute amount of formaldehyde that flowed through it increased 8-fold within the first half of the transition, and then decreased.

The authors generated a mathematical model based on known reaction rates from the short and long pathways. When they simulated methanol exposure, the model predicted a switch from long to short pathway very similar to what they had observed experimentally.

The authors conclude that the pathways are not in fact redundant, but fulfill different functions. The long pathway is not an efficient means of serine synthesis from formaldehyde; in fact, the small amount of serine it produces is at some energy cost. But it allows the cell to spend ATP—the molecular fuel—to jump-start formaldehyde assimilation while avoiding formaldehyde accumulation when the cells first experience methanol. The short pathway is a direct and efficient (energy-free) route to serine production (and hence growth), but one that is slower to reach its cruising speed. Thus, the cells use these two formaldehyde assimilation routes like a driver uses the transmission of a car: start with powerful low gears when first accelerating, and shift to more efficient gears once hurtling down the road.

The combination of both pathways represents an elegant solution to the problem of growth in toxic environments and provides a useful paradigm for detoxification in medical and environmental contexts.

Marx CJ, Van Dien SJ, Lidstrom ME (2005) Flux analysis uncovers key role of functional redundancy in formaldehyde metabolism. DOI: 10.1371/journal.pbio.0030016

DNA Recombination and Repair—A New Twist to RecA Function

DOI: 10.1371/journal.pbio.0030070

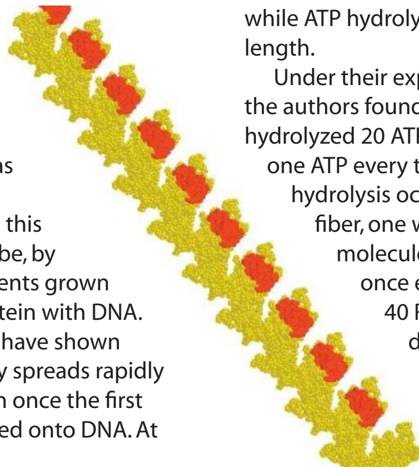
Molecular motors harness the energy of ATP (or GTP, a related energy currency) and transform it into mechanical force. Well-known examples of motors include myosin and dynein, proteins that use ATP to ferry intracellular cargo along fibers made of actin or tubulin proteins. The ATP-dependent assembly of actin or tubulin fibers itself can work as a motor: for instance, the march of white blood cells toward pathogens is powered by the growth of actin filaments pushing against the cells' membranes. In all cases, coherent motion implies a coordinated and polarized use of energy.

Now, Julia Cox, Oleg Tsodikov, and Michael Cox present evidence indicating that filaments of the bacterial RecA protein, long known for their role in homologous recombination and DNA repair, have properties reminiscent of a molecular motor as well. RecA filaments consist of DNA helices lined with RecA protein. RecA filaments invade a region of double-stranded DNA with similar nucleotide sequence, displacing one strand to pair with the other. Strand invasion can lead to a re-assortment—known as recombination—of DNA regions on either side of the shared sequence. It can also initiate the repair of DNA lesions during replication—the process by which a DNA molecule is copied to make two.

RecA is also an ATPase, an enzyme capable of hydrolyzing (breaking down) ATP, when bound to DNA. RecA uses ATP to carry out strand exchange over long sequences and impose direction to the exchange, to bypass short sequence heterogeneities, and to stall replication so DNA lesions can be mended. But how

RecA molecules within a filament coordinate and organize their activities to carry out these functions has remained obscure.

Cox et al. addressed this problem in the test tube, by examining RecA filaments grown from mixing RecA protein with DNA. Previous experiments have shown that filament assembly spreads rapidly in the 5'-to-3' direction once the first RecA molecule is loaded onto DNA. At the same time, ATP hydrolysis causes the release of RecA from DNA, but the exact rate of RecA dissociation is not known. Experiments suggest, however, that RecA molecules only dissociate from DNA when they are at the fiber's 5' end, while ATP hydrolysis occurs all along its length.



DOI: 10.1371/journal.pbio.0030070.g001

A RecA filament with every sixth molecule in red

Under their experimental conditions, the authors found that a RecA molecule hydrolyzed 20 ATPs per minute—or one ATP every three seconds. If ATP hydrolysis occurred randomly in the fiber, one would expect a RecA molecule to dissociate about once every 1.5 seconds, or 40 RecA molecules to dissociate per minute. But this is not what the authors found—instead they estimated the dissociation to be at a rate of 120 RecA molecules per minute. Hence, six RecA molecules dissociate from a fiber in the time—three seconds—it takes for an individual RecA molecule to burn one ATP. This implies a filament organization in which every RecA molecule hydrolyzes ATP in synchrony with the sixth RecA molecule to its left and the sixth RecA molecule to its right.

The authors note that there are approximately six RecA molecules per helical turn in a RecA filament. They propose that the RecA molecules hydrolyzing ATP at any given moment are aligned in a "stripe" that runs along the side of the filament. This stripe of ATP hydrolysis moves around the fiber in a repeating pattern of six steps. At the 5' end of the fiber, ATP hydrolysis leads to RecA release. But in the middle of the fiber, it could work as a rotary motor, with the power to wind or unwind DNA and drive strand invasion through difficult passages of damaged DNA.

Cox JM, Tsodikov OV, Cox MM (2005) Organized unidirectional waves of ATP hydrolysis within a RecA filament. DOI: 10.1371/journal.pbio.0030052

Unique Double-Barreled Enzyme Makes Methionine the Hard Way

DOI: 10.1371/journal.pbio.0030054

If a cell is a complex symphony of chemical reactions, its enzymes are the instruments through which this elemental music is played. Each reaction is catalyzed by a specific enzyme, whose uniquely shaped active site not only binds reactants, but, by forming weak and temporary bonds, coaxes them into new orientations with new partners, thus creating the products. Determining exactly how any individual enzyme accomplishes this task—which amino acids make up the active site, which bonds form where when enzyme meets substrate, which electrons switch partners as new bonds form—is the work of the structural biochemist. In this issue, Martha Ludwig and Robert Pejchal elucidate the structure of cobalamin-independent methionine synthase (MetE) from the bacterium *Thermotoga maritima*, and describe how it catalyzes the formation of the amino acid methionine.

Methionine synthases actually come in two forms, which use somewhat different mechanisms to accomplish the same task: transfer of a methyl group (CH_3) from methyltetrahydrofolate to the terminal sulfur of homocysteine. The cobalamin-dependent form, MetH, relies on the cofactor cobalamin (vitamin B12), which pulls the methyl away at one active site, and then donates it at a second active site. Here, a central zinc atom binds and activates homocysteine, enabling it to attack the incoming methyl group that is attached to cobalamin. MetE, on the other hand, has no cofactor and only one active site, which sits at the junction of two eight-stranded barrels. The structure and sequence of these barrels indicate they arose through duplication of a primordial zinc-bearing, homocysteine-binding protein. This unique duplex now bears only one zinc atom, deep within the cleft separating the two barrels.

As in MetH, the role of the zinc is to bind homocysteine, but in MetE, this event also induces a conformation change around the zinc. The zinc and its coordinating partners form an umbrella; entering from the handle end, the homocysteine sulfur pulls the zinc toward it and turns the umbrella inside out. Methyltetrahydrofolate initially binds along the edge of the cleft, with the methyl group on the folate oriented far from the sulfur on the homocysteine, as can be seen in the research article's Video S1 (DOI: 10.1371/journal.pbio.0030031.sv001). There must be subsequent conformational changes within the active site that serve to bring the two substrates together and promote transfer of the methyl group. Exactly how methyltetrahydrofolate reorients within the cleft to complete the reaction is not yet clear. The reaction catalyzed by MetE proceeds slowly, at only 1%–2% of the speed of that catalyzed by MetH. One reason for this rather sluggish activity is that homocysteine, even when activated by binding to zinc, is much poorer than cobalamin at displacing the methyl group of methyltetrahydrofolate.

While MetE's unique active-site structure was made possible by gene duplication, the two barrels are no longer identical. Through evolution, the second, N-terminal, barrel has lost the ability to bind zinc or homocysteine, and indeed appears to contribute little to the active function of the enzyme. Nonetheless, this barrel may be necessary to temporarily isolate the substrates from solvent and to form the hydrophobic environment in which the reaction is more favorable. Further research may indicate more about the function of this unequal partner, and provide more detail on the exact atomic movements within the cleft at the moment of reaction.

Pejchal R, Ludwig ML (2004) Cobalamin-independent methionine synthase (MetE): A face-to-face double barrel that evolved by gene duplication. DOI: 10.1371/journal.pbio.0030031

What Does an Airline Traveler Have in Common with a Glowing Fish?

DOI: 10.1371/journal.pbio.0030046

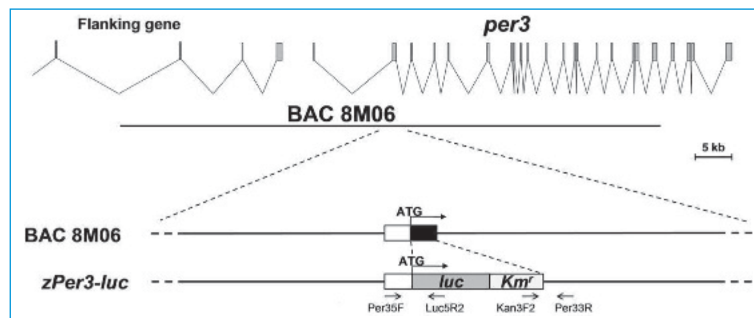
In William Gibson's novel *Pattern Recognition*, the protagonist posits a theory of jet lag: "Souls can't move that quickly, and are left behind, and must be awaited, upon arrival, like lost luggage."

Science has yet to address the issue of a spiritual speed limit, but it is generally accepted that jet lag actually results from the upset of the body's circadian clock, a biochemical pacemaker that dictates daily rhythms in sleep and wakefulness as well as body temperature and metabolic activity. In humans, the circadian rhythm responds to many factors, but daytime–nighttime (or, more precisely, light–dark) cycles are one major regulator. It is possible to gradually reset an upset circadian clock; if travelers remain in the same place for long enough, their circadian rhythm will eventually adjust to the new time zone and ambient light patterns, and the symptoms of jet lag will disappear.

The more scientists know about the workings of the circadian clock, the closer they can come to manipulating it. Much is known about the molecular machinery of the circadian clock in the fruitfly, *Drosophila melanogaster*. Two circadian proteins, Clock and Cycle, cooperate to induce expression of two other proteins, Per and Tim, and when levels of Per and Tim are high enough, they cooperate to shut off their own expression. This negative feedback loop leads to periodic fluctuations in the level of Per and underlies the circadian rhythm in flies. However, until recently, not much was known about the mechanics of the circadian clock in vertebrates.

Maki Kaneko and Gregory Cahill have created a new tool for investigating the components of the circadian clock in vertebrates: a zebrafish that luminesces (glows) in sync with the periodicity of its circadian clock. To do this, the researchers created a transgene that places expression of the firefly *luciferase* gene under the control of the promoter of the zebrafish circadian gene *period3 (per3)*. Each cell of the transgenic fish has one normal copy of the *per3* gene and one copy of the *period3-luciferase* fusion gene *(per3-luc)*. Therefore, whenever *per3* expression is normally turned on in a cell, the cell produces Per3 protein and also produces the luciferase protein.

While characterizing their transgenic zebrafish, the authors made some interesting findings. First, contrary to earlier studies, the authors found that *per3* periodicity is not hardwired into zebrafish embryos; instead, *per3* periodicity is entrained by alternating light–dark cycles, which must occur at specific stages in early development. Also, other external factors such as ambient temperature can influence both the level of *per3* mRNA expressed in the animal and the magnitude of its protein-level oscillations. Because the establishment of circadian rhythms in the adult animal can be so strongly influenced by conditions experienced by the embryos, the authors suggest using a standardized set of conditions for the culture of transgenic embryos in future experiments involving adult fish.



DOI: 10.1371/journal.pbio.0030046.g001

A fusion gene (period3-luciferase) was used to track circadian rhythms

Under these controlled conditions, Kaneko and Cahill anticipate that these transgenic zebrafish will be quite useful in examining the molecular machinery of the vertebrate circadian clock. For example, researchers can use the *per3-luc* transgenic zebrafish in forward genetic screens (where researchers mutagenize the animal to induce a desired phenotype and then identify the mutated gene responsible for the phenotype). In this case, mutagenized zebrafish could be examined for disruptions of *per3-luc* periodicity or expression. What is more, luminescence can be measured quickly and noninvasively, making this animal an ideal candidate for high-throughput screening aimed at identifying components of the circadian clock in the zebrafish. Thanks to luminescent fish, scientists may someday gain enough insight to make jet lag a thing of the past.

Kaneko M, Cahill GM (2005) Light-dependent development of circadian gene expression in transgenic zebrafish. DOI: 10.1371/journal.pbio.0030034

Rice Genome Approaches Completion

DOI: 10.1371/journal.pbio.0030047

In April 2002, *Science* published draft genome sequences for the two major subspecies of cultivated rice, *Oryza sativa*. The release of the rice genome—the first plant crop to be sequenced—was big news. Rice is a staple crop for more than half of the world's population, and it was hoped that the availability of the genome sequence might enable scientists to develop more productive rice strains or strains that are more environmentally friendly. Furthermore, the rice genome might provide the key to understanding the genetics of other major cereal crops, all of which have much larger genomes.

But the sequences published in 2002 were only draft genomes, containing many gaps and errors—works-in-progress rather than finished products. Now, a large group of scientists led by the Beijing Institute of Genomics is publishing a much improved, near-complete genome analysis of the *indica* and *japonica* subspecies of *O. sativa*, which are eaten in India and China, and Japan, respectively. Their analysis team, led by Gane Ka-Shu Wong, provides important insights into the evolution of rice.

First of all, the team improved their original whole-genome shotgun sequencing of *indica* by generating significantly more sequence data, and then they used better methods to assemble these data. In whole-genome shotgun sequencing, the entire genome is chopped into random fragments, each fragment is sequenced, and then powerful computer programs search for overlaps and put all these data in order. It's like putting a fiendishly difficult jigsaw puzzle together by looking for patches of matching color.

The key to the improvement in the genome sequence analysis is that the researchers have used the combined DNA sequence data from the two subspecies to facilitate the sequence assembly. The result is a nearly 1,000-fold increase in contiguity for the two genome sequences. In other words, while the original draft was very fragmented, in the new version, 97.7% of the genes can be found, in either the *indica* or the *japonica* dataset, on one piece of DNA whose position along the chromosomes is well defined.

The researchers have also used their improved genome sequence to investigate the evolutionary history of rice. Central to evolution is the development of new functions through mutation of existing genes. But when mutations occur in functional genes, the result is rarely beneficial, so it is thought that evolution is more likely to proceed first by duplicating existing genes and then experimenting on the "backup" copy of the gene. Wong and colleagues report that there is evidence in the rice DNA sequences for a whole-genome duplication event just before the grasses diverged from other flowering plants, about 55–70 million years ago. This genome duplication may have played a role in the origin of the grasses, which then spread rapidly across the world to provide important sources of food that, among other things, possibly influenced human evolution.

DOI: 10.1371/journal.pbio.0030047.g001

A bowl of *indica* (white, long grains) and *japonica* (brown, short grains) rice

Analysis of the rice genomes also indicates that a small chromosomal segment was duplicated about 21 million years ago and that there is massive ongoing duplication of individual genes. These individual gene duplications provide a continuous source of raw material for gene genesis and very likely contribute to the differences between members of the grass family. Now the challenge is to use the rice sequences as a basis for detailed genetic analyses of additional cereal crops and for the development of improved strains of not only rice, but wheat, maize, and other important food crops.

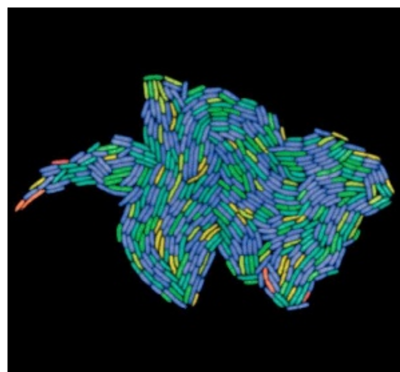
Yu J, Wang J, Lin W, Li S, Li H, et al. (2005) The genomes of *Oryza sativa*: A history of duplications. DOI: 10.1371/journal.pbio.0030038

Aging and Death in *E. coli*

DOI: 10.1371/journal.pbio.0030058

As human beings, aging and death are an inevitable part of our lives. As we pass through each decade, the concrete signs of aging—greying hair, aches and pains, the gradual failure of one organ system after another—and the realization that we are mortal increasingly occupies our thoughts.

All other multicellular animals and plants also show clear signs of aging, as do some single-celled organisms. In the yeast *Saccharomyces cerevisiae* (baker's yeast), for example, the function of individual cells gradually declines with time, and each yeast cell has a finite life span. In organisms like this, it has been proposed that reproduction by asymmetric division is a prerequisite for aging. In other words, for a unicellular organism to age, when it divides, it must give rise to a "parent" cell and a smaller offspring cell (as in yeast), which then has to go through a juvenile phase of growth or differentiation before it divides. At each cell division, the parent cell becomes older until it reaches its natural life span and dies.



DOI: 10.1371/journal.pbio.0030058.g001

A growing microcolony of *E. coli*

But what about organisms that produce two apparently identical cells when they divide? Do such organisms age? The assumption has been for some years that cells that divide symmetrically do not age and are functionally immortal. Eric Stewart and colleagues have now tested this idea by analyzing repeated cycles of reproduction in *Escherichia coli*, a bacteria that reproduces without a juvenile phase and with an apparently symmetric division.

E. coli is a rod-shaped organism that reproduces by dividing in the middle. Each resultant cell inherits an old end or pole and a new pole, which is made during the division. The new and the old pole contain slightly different components, so although they look the same, they are physiologically asymmetrical. At the next division, one cell inherits the old pole again (plus a brand new pole), while the other cell inherits, a not-quite-so-old pole and a new pole. Thus, Stewart and co-workers reasoned, an age in divisions can be assigned to each pole and hence to each cell.

The researchers used automated time-lapse microscopy to follow all the cell divisions in 94 colonies, each grown from a single fluorescently labeled *E. coli* cell. In all, the researchers built up a lineage for 35,049 cells in terms of which pole—old or new—each cell had inherited at each division during its history. They found that the cells inheriting old poles had a reduced growth rate, decreased rate of offspring formation, and increased risk of dying compared with the cells inheriting new poles. Thus, although the cells produced when *E. coli* divide look identical, they are functionally asymmetric, and the "old pole" cell is effectively an aging parent repeatedly producing rejuvenated offspring.

Stewart and his colleagues conclude that no life strategy is immune to the effects of aging and suggest that this may be because immortality is too costly or is mechanistically impossible. This may be bad news for people who had hoped that advances in science might eventually lead to human immortality. Nevertheless, *E. coli* should now provide an excellent genetic platform for the study of the fundamental mechanisms of cellular aging and so could provide information that might ameliorate some of the unpleasantness of the human aging process.

Stewart EJ, Madden R, Paul G, Taddei F (2005) Aging and death in an organism that reproduces by morphologically symmetric division. DOI: 10.1371/journal.pbio.0030045

Loss of Sight and Enhanced Hearing: A Neural Picture

DOI: 10.1371/journal.pbio.0030048

Stevie Wonder and Ray Charles are often cited as evidence that blindness confers superior musical ability. Wonder lost his sight after an incubator-related oxygen overdose during infancy; Charles lost his as a boy to glaucoma. It's impossible to know whether sight would have compromised their success, but many gifted musicians, from Jose Feliciano to Rahsaan Roland Kirk, lost their sight at an early age.

A number of human studies show that blind persons perform nonvisual tasks better than those with sight. Neuroimaging studies of blind persons performing nonvisual tasks, including hearing, show activity in brain areas normally associated with vision. But much remains to be learned about the nature and extent of this phenomenon: how these "visual areas" are used, the mechanisms that generate individual differences (not all blind persons can localize sounds better than the sighted, for example), and the neural processes that underlie it.

The task of localizing sound—which requires integrating information available to one ear only (monaural sounds available, for example, when one ear is plugged) or information derived from comparing sounds binaurally—is particularly suited to investigating the neural remapping that seems to follow vision loss. In a previous study, Franco Lepore and colleagues showed that people who lost their sight at an early age could localize sound, particularly from monaural cues, better than those who could see. These findings suggested that areas of the brain normally dedicated to processing visual stimuli (the visual cortex, located at the back of the brain in the occipital lobe) might play a role in processing sound in these individuals. In a new report, Lepore and colleagues use functional imaging studies to investigate the functional relationship between neural activity and enhanced hearing abilities in the blind, and find a strong correlation between superior sound localization skills and increased activity in the brain's visual center.

The authors hypothesized that if visual cortex recruitment bolstered auditory function in some individuals, then visual cortex activity would correlate with individual differences in performance, and the degree of activity should predict such differences. Nineteen people—seven sighted and twelve who lost their sight at an early age—were placed in an echo-free chamber and asked to indicate where a sound was coming from, using either one (monaural) or both (binaural) ears. The participants then performed the same tasks within a positron emission tomography (PET) machine, which measures brain activity through changes in cerebral blood flow (CBF).

Five of the blind participants could accurately localize sounds monaurally; most of the sighted could not. (All 19 participants had no trouble localizing binaural sounds.) Only the blind individuals with superior localization skills showed increased CBF in the visual cortex while performing monaural localization tasks. Interestingly, during binaural localization, the sighted participants showed decreased CBF in visual cortical areas. This decrease comports with previous studies showing that engaging one brain center—say, the temporal lobe, which processes sound—inhibits activation of others—such as the occipital lobe, which processes visual cues. These inhibitions appear to be absent in blind persons, though it's not clear why. It could be that blind persons don't need such inhibitions, the authors speculate, or maybe unrestricted access to the visual center serves to compensate for vision loss by boosting nonvisual senses.

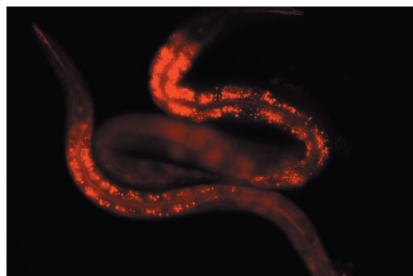
Whether the enhanced auditory performance reported here simply reflects increased efficiency of auditory processing or indicates "supranormal" powers, Lepore and colleagues argue that their results show that the visual cortex is "specifically recruited to process subtle monaural cues more effectively." It will be interesting to learn whether blind persons can recruit visual centers for other auditory tasks or to help them navigate the world without sight. Such studies would be vital for tailoring sensory support to suit individual needs and maybe even suggest ways to facilitate the neural cross talk that enhances auditory performance. But don't expect such innovations to recreate the likes of Rahsaan Kirk or Ray Charles anytime soon.

Gougoux F, Zatorre RJ, Lassonde M, Voss P, Lepore F (2005) A functional neuroimaging study of sound localization in early-blind individuals. DOI: 10.1371/journal.pbio.0030027

Opposing Fat Metabolism Pathways Triggered by a Single Gene

DOI: 10.1371/journal.pbio.0030083

Regulating metabolism of fat is an important challenge for any animal, from nematodes to humans. Central players in the regulatory network are the nuclear hormone receptors (NHRs), which are transcription factors that turn on or off a set of target genes when bound by specific lipid molecules. NHR genes number 48 in mammals, and a surprising 248 in nematodes. Despite the difference in quantity, there are some structural similarities between NHRs in these two groups, in particular, between the nematode gene *nhr-49* and the mammalian *HNF4*. In this issue, Keith Yamamoto and colleagues show that *nhr-49* controls two different aspects of fat metabolism, which interact to form a feedback system controlling the consumption and composition of fats in the nematode.



DOI: 10.1371/journal.pbio.0030083

Regulation of fat content and lifespan in *C. elegans*

Using RNAi to suppress gene expression, the researchers discovered that when *nhr-49* was absent, the lifespan of the nematode was reduced by more than 50%, and the animal displayed numerous gross abnormalities in the gut and gonad. This was accompanied by unusually high fat content in the larvae. By using quantitative PCR to measure output of fat and glucose metabolism genes, the researchers showed that deletion of *nhr-49* changed expression of 13 of these genes, with the most dramatic effects occurring within two metabolic pathways: mitochondrial lipid oxidation and fatty acid desaturation.

Oxidation degrades lipids to release energy, explaining the build-up of fat in *nhr-49*-suppressed larvae. One of *nhr-49*'s normal functions is to increase expression of the mitochondrial acyl–Coenzyme A (CoA) synthetase gene *acs-2*. A

principal role for mitochondrial acyl-CoA synthetases is to "activate" free fatty acids for transport into mitochondria, where they are oxidized. This process involves attaching a CoA group to a free fatty acid, and often serves as a rate-limiting step in lipid oxidation. Indeed, the authors found that suppression of *acs-2* alone was sufficient to reproduce the high-fat phenotype, while overexpression of *acs-2* rescued the phenotype even in the absence of *nhr-49*.

Fatty acid desaturation is the process of converting saturated fats into unsaturated ones, by forming one or more double bonds between adjacent carbons in the tail. This process is catalyzed by fatty acid desaturase enzymes. *nhr-49* increases expression of several desaturases, most importantly *fat-7*, which converts stearic acid to oleic acid; deletion of *nhr-49* more than doubled the proportion of stearic acid compared to oleic acid.

RNAi interference of *fat-7* alone produced two interesting results. First, it shortened the nematode life span, suggesting this was the primary pathway through which *nhr-49* suppression exerted that same effect. Second, it produced some effects that were opposite those of *nhr-49* suppression: specifically, it reduced rather than increased fat content, and it increased rather than reduced expression of *acs-2*.

These results show that in its normal actions, *nhr-49* sets in motion two opposing pathways: it increases *acs-2*, which leads to reduction of fat content, and it increases *fat-7*, which, by reducing *acs-2*, increases fat content. Surprisingly, this behavior links *nhr-49* most closely not to *HNF4*, with which it shares the most structural similarity, but to another type of mammalian NHR, called peroxisome proliferator-activated receptors (PPARs). Further investigation of this link may lead to better understanding of the functions of PPARs, and provide opportunities for altering their function for treatment of fat metabolism disorders such as diabetes and obesity.

Gilst MR, Hadjivassiliou H, Jolly A, Yamamoto KR (2005) Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. DOI: 10.1371/journal.pbio.0030053

The Bottleneck of Central Processing: Clues from Reaction Times

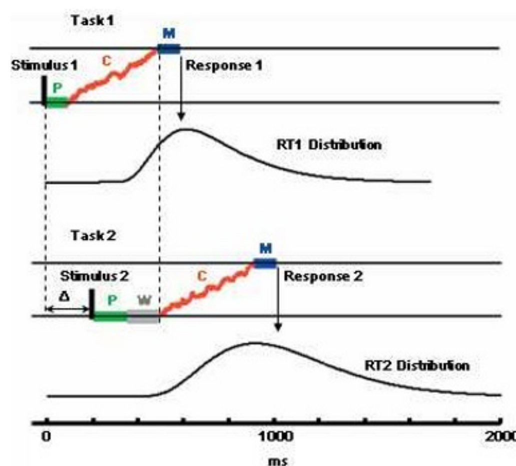
DOI: 10.1371/journal.pbio.0030084

Between stimulus and response lies a black box—the mind—whose inner workings are largely unmapped. One of the essential questions about those inner workings concerns the serial versus parallel nature of their processing capabilities. Parallel processing allows multiple tasks to proceed at once, while serial processing creates a bottleneck through which multiple tasks must pass, one at a time. Any reasonably complex task is likely to involve both parallel and serial components, and parsing a task into its components is a central goal for researchers of cognitive processing. In a new study, Mariano Sigman and Stanislas Dehaene propose a model of cognitive processing for a set of simple tasks in which a bottleneck occurs between initial sensory processing and motor response. They predict that this bottleneck will contribute significantly to variations in response time as the cognitive challenge increases and verify this by testing people on a specific numerical evaluation task.

A simple but highly effective technique for examining bottlenecks is to measure variations in response time for a task as the stimulus is varied in some small but cognitively challenging way, or when the stimulus is presented along with a stimulus for a competing task. The task in this study was to determine whether a presented number was greater than or less than 45. The complexity of the task was determined by three variables: notation, distance, and response complexity. Notation was varied by presenting the number either as a numeral or its spelled-out equivalent (for instance, "36" or "thirty-six"). Distance was varied by presenting numbers either closer to or further from 45 (for instance, 31 versus 36), and the required response was either one or two finger taps. In a separate series of experiments, the researchers challenged the subject with an interfering tone-recognition task at the same time as or slightly after the presentation of the numerical task.

The use of these two sets of experiments allowed the authors to deduce two different kinds of information about the processing involved in the number task. First, by varying the delay in presentation of the tone-interference task and measuring the delay in response time for the number task, they showed that both number perception and motor response can proceed in parallel with other, competing tasks, while the central component of the number task—determining distance—was processed serially, through a central bottleneck.

Next, Sigman and Dehaene turned off the tone, and asked how variations in notation, distance, and response complexity altered the variance in the response time—that is, the spread of values for the same task by the same subject. For all three variables, the more challenging task (numbers presented as words, smaller distance, or two taps) had an increased response time. However, only the calculation of distance increased the spread of values obtained in multiple trials. This further suggests that only the central calculation step—what the authors refer to as stochastic integration—proceeds through a central bottleneck, while the other two components can be processed in parallel. Thus, in both experiments, the task was parsed by the brain into the same components, with the serial component being the one subject to the most variance.



DOI: 10.1371/journal.pbio.0030084

Comparing human reaction times

Sigman and Dehaene note that the ability of the perceptual and motor parts to be performed without central computation depends on the extreme simplicity of the tasks in this experiment. More complex motor challenges, for instance, undoubtedly would require some central input, and thus proceed through the bottleneck. Similarly, a high degree of training in the numerical distance task would likely increase the automaticity of the response, thus avoiding the central slowing seen in the task-naïve subjects studied here.

Sigman M, Dehaene S (2005) Parsing a cognitive task: A characterization of the mind's bottleneck. DOI: 10.1371/journal.pbio.0030037