Research Digest

Synopses of Research Articles

Gene Targeting Turns Mice into Long-Distance Runners

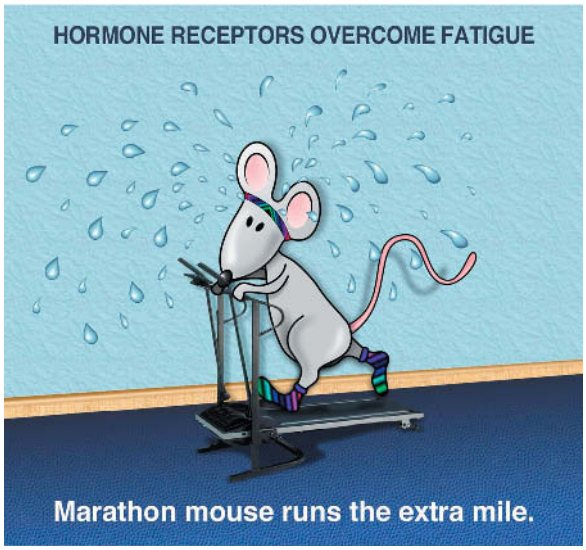
DOI: 10.1371/journal.pbio.0020322

Have you ever noticed that long-distance runners and sprinters seem specially engineered for their sports? One's built for distance, the other speed. The ability to generate quick bursts of power or sustain long periods of exertion depends primarily on your muscle fiber type ratio (muscle cells are called fibers), which depends on your genes. To this extent, elite athletes are born, not made. No matter how hard you train or how many performance-enhancing drugs you take, if you're not blessed with the muscle composition of a sprinter, you're not going to break the 100-meter world record in your lifetime. (In case you'd like to try, that's 9.78 seconds for a man and 10.49 seconds for a woman.)

Of course that doesn't prevent those at the highest levels from using the latest performance enhancer to get that extra 1% edge. But wait until trainers hear about the Marathon Mouse. A new study by Ronald Evans and colleagues provides evidence that endurance and running performance can be dramatically enhanced through genetic manipulation.

Skeletal muscles come in two basic types: type I, or slow twitch, and type II, or fast twitch. Slow-twitch fibers rely on oxidative (aerobic) metabolism and have abundant mitochondria that generate the stable, long-lasting supplies of adenosine triphosphate, or ATP, needed for long distance. (For more on muscle fiber metabolism, see synopsis titled "A Skeletal Muscle Protein That Regulates Endurance") Fast-twitch fibers, which produce ATP through anaerobic glycolysis, generate rapid, powerful contractions but fatigue easily. Top-flight sprinters have up to 80% type II fibers while long-distance runners have up to 90% type I fibers. Coach potatoes have about the same percentage of both.

Endurance training can enhance the metabolic performance of muscle types, and now it appears that training can also induce conversion between fiber types. Specific changes in gene expression trigger this oxidative fiber transformation, but the transcription factor responsible for engineering this shift was unknown. Evans and colleagues suspected that a nuclear receptor called PPARδ—a major regulator of fat burning in fat tissue that is also prevalent in skeletal muscle—might be involved.



DOI: 10.1371/journal.pbio.0020322.g001

To investigate this possibility, the authors genetically engineered mice to express an activated form of the PPARδ protein in skeletal muscle. Type I fibers normally express higher levels of PPARδ than type II fibers, and the transgenic mice showed much higher levels of the protein than their normal littermates. The transgenic mice also had much redder muscles than the controls—type I fibers have high levels of myoglobin, the red-pigmented protein that facilitates the movement of oxygen within muscle—and elevated levels of proteins associated with mitochondrial biogenesis and operation. A final line of evidence indicating a type I fiber switch was the elevated level of specialized type I contractile proteins and decreased level of specialized type II contractile proteins in the transgenic mice. Interestingly, these same results were seen when naturally occurring (endogenous) PPARδ levels were stimulated in the normal mice (with an orally active compound). This suggests that muscle fibers can be transformed into type I endurance fibers by simply activating the endogenous PPARδ pathway.

In a weight-conscious world, oxidative fibers are thought to offer resistance against obesity since obese individuals have fewer type I fibers than average-weight individuals. Sure enough, transgenic mice fed a high-fat diet gained far less weight than normal mice fed the same diet, even in the absence of exercise. The transgenic mice had much smaller fat cells, which the authors attribute to enhanced oxidative capacity of the muscle tissue, and improved glucose tolerance. (Obese individuals lose the ability to metabolize glucose, which puts them at risk for diabetes.) But what about performance? Remarkably, the marathon mice ran about an hour longer than controls, showing dramatic improvement in both running time and distance—increases of 67% and 92%, respectively.

Altogether, these results show that PPARδ drives the conversion of type I muscle fibers by activating pathways that enhance physical performance and protect against obesity. The finding that endurance and running capacity can be genetically manipulated suggests that muscle tissue is far more adaptable than previously thought. Maybe Olympiads can be made after all—but don't give up on training just yet. A full understanding of the molecular basis of muscle fiber determination, including the interactions between PPARδ and its regulatory components, awaits further study.

Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, et al. (2004) Regulation of muscle fiber type and running endurance by PPARδ. DOI: 10.1371/journal.pbio.0020294

A Skeletal Muscle Protein That Regulates Endurance

DOI: 10.1371/journal.pbio.0020315

It's a common runner's complaint. Just when you've built up enough strength and endurance to make running fun, those niggling aches and pains won't go away. Every time your foot hits the ground, a force equal to about twice your weight shoots through your body, eventually chipping away at bones, cartilage, muscles, tendons, ligaments, and joints. For those lucky souls who can take the pounding, the main limitation to running performance stems from muscle fatigue. Now, Randall Johnson and colleagues report that a protein found in skeletal muscle profoundly influences muscle endurance.

Running, like any sustained skeletal muscle activity, consumes large quantities of adenosine triphosphate (ATP), a molecule that fuels many essential cell processes. A number of metabolic pathways supply muscle tissue with the ATP needed to power muscle contraction and sustain ongoing exercise. Which pathway predominates depends on factors like speed, duration, and type of activity, as well as on the availability of oxygen, which fluctuates during activity. (For more on muscle cell type and endurance, see the synopsis titled "Gene Targeting Turns Mice Into Long-Distance Runners.")

Say you start a half-hour run with a sprint. Within a few seconds, your body uses up the oxygen in its muscles and has to switch to anaerobic pathways, which metabolize sugars and fats to regenerate ATP. Aerobic pathways operate inside mitochondria, the cell's major power generators. Anaerobic pathways like glycolysis function in the cytoplasm.

Hypoxia (the physiological state that occurs when oxygen levels drop below normal levels) governs how ATP is recycled and which energy-producing substrates (for example, glucose or fatty acids) are used; it also generates metabolic by-products, like lactic acid, during strenuous exercise. (Runners know the "lactic acid burn" associated with reduced blood pH.) Glycolysis—the primary source of anaerobic energy in animals—uses glucose, stored as glycogen in muscle cells, to produce ATP. When blood oxygen levels drop, the gene transcription factor hypoxia-inducible factor 1α (HIF-1α) triggers the glycolytic pathway.

To understand how HIF-1α regulates skeletal muscle function, Johnson's team generated mice that couldn't express HIF-1α in skeletal muscle. Normal and mutant mice went through exercise routines that included swimming and running on treadmills. After exercise, the normal mice had increased levels of gene transcripts and enzymes involved in glucose transport and metabolism. In the mutant mice, expression of these glycolysis-associated genes and enzymes was significantly lower. The mutants' ATP levels, however, were normal. Without the molecular machinery to engage anaerobic metabolism, their muscles switched to aerobic pathways. The presence of enzymes that respond to reduced ATP levels by increasing mitochondrial ATP production, combined with low levels of lactic acid, confirmed the switch.

During endurance tests, the mutants could swim and run uphill (on treadmills tilted upward) longer than the normal mice, but when it came to running downhill, the normal mice prevailed. Downhill running, it turns out, favors glycolytic metabolism; uphill running and swimming favor oxidative pathways, which the mutants were predisposed toward. But their inappropriate use of this pathway came at a cost. By the final day of a four-day exercise routine, the mutants' run time was significantly shorter and their muscles were clearly damaged.

The mutants displayed a number of the trademark muscle defects seen in human patients with glycolytic processing disorders. These patients often have reduced lactate levels and elevated levels of mitochondrial enzymes, which apparently can cause a second wind and enhance endurance. This inappropriate use of oxidative pathways—which compensates for the inability to trigger glycolysis—may account for the exercise-induced muscle damage associated with these diseases.

These results demonstrate that losing the molecular wherewithal to engage hypoxia response pathways has serious consequences for muscle function during exercise; it can give increased endurance, but at a high price. The mouse model presented here will help researchers explore how muscles normally function in response to low oxygen and how metabolic deficiencies cause debilitating muscle disease.

Mason SD, Howlett RA, Kim MJ, Olfert M, Hogan MC, et al. (2004) Loss of skeletal muscle HIF-1α results in altered exercise endurance. DOI: 10.1371/journal.pbio.0020288

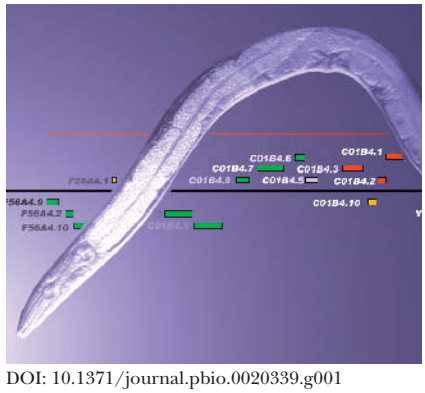
A Novel Pathway for a Tumor Suppressor

DOI: 10.1371/journal.pbio.0020339

Millions of different proteins exist in nature, each with a unique structure that determines its function. Proteins can have different effects depending on where they are in the cell and which proteins or pathways they associate with. The number of proteins produced by a cell varies, but scientists estimate that the human genome produces some 100,000 proteins, and with thousands of proteins likely to be active in a single cell, it's inevitable that the molecular components of cellular pathways overlap. It is thought that this may be the case for a tumor suppressor named VHL (after its role in von Hippel-Lindau disease, an inherited cancer syndrome that predisposes affected individuals to kidney and vascular tumors).

The broad strokes of VHL action have been outlined: VHL is a ubiquitin ligase, an enzyme that targets proteins for destruction. VHL's best characterized target is a transcription factor called hypoxia-inducible factor 1 (HIF-1). When oxygen levels drop below normal—a condition called hypoxia—HIF-1 proteins are not degraded and may enter the nucleus, where they trigger the transcription of roughly 100 genes whose proteins either increase oxygen delivery or engage metabolic pathways that help the cell adapt to hypoxia. Scientists have long suspected that VHL has other targets, yet only HIF-1 has been clearly established. Now Peter Ratcliffe and colleagues use genetic methods in the nematode *Caenorhabditis elegans* to provide direct evidence of a HIF-1-independent function of VHL.

Previous studies have shown that when cells lack VHL proteins, HIF-1 is not



DOI: 10.1371/journal.pbio.0020339.g001

Genetic evidence for HIF-independent VHL-regulated pathways

degraded, resulting in the overexpression of HIF target genes. VHL-defective cells also show abnormalities in the extracellular matrix, the structural scaffolding that surrounds the cell. But it has not been clear whether these effects stem from HIF-1 dysregulation or something else.

To disentangle the actions of the two proteins, Ratcliffe and colleagues compared the consequences of VHL protein inactivation in a variety of genetic backgrounds in *C. elegans*. Then they analyzed the gene expression profiles of each of these mutant strains to identify pathways that required VHL but not HIF-1. To their surprise, the authors found, "all of the VHL-regulated genes fell into one of two patterns." Their expression was either independent of HIF-1 and dependent on a range of genes associated with the extracellular matrix, or vice versa. What's more, these gene sets fell into distinct categories based on their chromosomal location, predicted functional similarities, and pattern of dysregulation.

These results, Ratcliffe and colleagues conclude, reflect the disruption of "two discrete aspects of VHL function." One depends on HIF-1—inhibiting the transcription factor when oxygen concentrations are normal—and one doesn't; disruption of this HIF-1-independent function produces defects similar to those seen in mutants with defects in extracellular matrix assembly. These results also fall in line with other studies that have linked VHL deficiency to extracellular matrix defects, though the precise link remains unclear. It's also not clear whether this HIF-1-independent function means that VHL is still functioning as a ubiquitin ligase but targeting a different substrate or whether it represents a completely different function of VHL. For now, direct evidence of a HIF-1-independent pathway for VHL charts a clear path for researchers interested in pinning down the functions of this undoubtedly multidimensional ubiquitin ligase. It also gives VHL syndrome researchers—who have long suspected that other functions of the VHL tumor suppressor play a role in the onset of the disease—a promising lead to explore.

Bishop T, Lau KW, Epstein ACR, Kim SK, Jiang M, et al. (2004) Genetic analysis of pathways regulated by the von Hippel-Lindau tumor suppressor in *Caenorhabditis elegans*. DOI: 10.1371/journal.pbio.0020289

Key to Cholesterol's Role in Nematode Development

DOI: 10.1371/journal.pbio.0020345

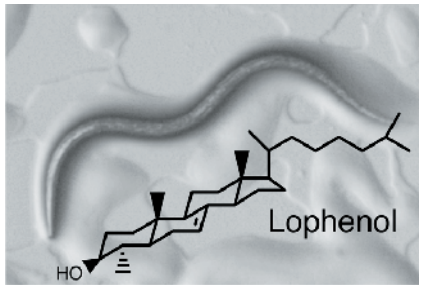
Cholesterol has a bad rap for its association with human heart disease. But actually cholesterol and other sterols are essential for a wide variety of organisms. For most eukaryotes—organisms whose cells have nuclei—sterols reside in the cell membrane and play major structural roles. Sterols keep cell membranes flexible, for example. These chemicals also hinder leakage of ions across the membrane, which is crucial in order for muscles to contract and nerves to conduct signals.

For the tiny (eukaryote) nematode worm *Caenorhabditis elegans,* sterols are a dietary staple. Worms can't make these chemicals from scratch, just as humans can't make vitamin C or the essential amino acids, so they have to harvest these chemicals from their surroundings. If nematodes hit hard times—they can't find enough sterols, say, or are starved or overcrowded—they can delay developing into adults. Instead, they enter a stage called a dauer in which they don't eat and hardly move a muscle. In this state, they can persist several months—many times their normal lifespan—and then revive when conditions improve.

Though *C. elegans* is extensively studied, there's still controversy over the role of cholesterol in this organism. To develop into adults, the nematodes need only small amounts of cholesterol in their diet, suggesting cholesterol does not play a major role in their membranes. Instead, nematodes—like many other eukaryotes—might use cholesterol to make hormones, which are typically active at very low concentrations. Such hormones could play a key role in the worms' development into either adults or dormant dauers. But no one had found any nematode hormones derived from cholesterol—until now.

In this issue of *PLoS Biology,* Teymuras Kurzchalia and colleagues show definitively that cholesterol does not play an essential structural role in *C. elegans*. Rather, cholesterol is the precursor for a hormone—or set of hormones—key in the worms' development into adulthood and thus key for reproduction. The researchers have partially purified this cholesterol derivative and named it gamravali, from the Georgian word for reproduction, "gamravleba."

When on sterol-free diets, all larvae showed arrested development, becoming dormant dauers. But, surprisingly, the concentration of cholesterol they needed to develop into adults was miniscule, around 20 nanomoles. When given scant amounts of cholesterol, the worms converted some of it to a sterol called lophenol. The researchers found, however, that supplementing a sterol-free diet with lophenol was not enough to sustain development into adulthood. Apparently the worms need cholesterol, which is fed into two distinct pathways: one makes lophenol and another makes the hormone gamravali.



DOI: 10.1371/journal.pbio.0020345.g001

The researchers have only partially purified gamravali, so they don't yet know its molecular weight or composition or even whether it is a single molecule. But by working with mutant worms, they have begun to pin down where gamravali acts in the worms' developmental pathway. One mutant *C. elegans* line, for instance, was unfazed by the cholesterol-free diet. These mutants were missing the *daf-12* gene, one of a set of genes crucial in nematode development and aging. On the cholesterol-free, lophenol-supplemented diet, these mutants developed into normal adults. Other mutant lines that each lacked one of several other *daf* genes, however, developed into dauers when deprived of cholesterol. In this way the researchers found where gamravali acts in the worms' developmental pathway: the hormone gamravali likely comes into play before *daf-12,* but after the other *daf* genes. Kurzchalia and colleagues are currently working to further purify gamravali and identify exactly how it gives cholesterol such a crucial role in the worms' lifecycle.

Matyash V, Entchev EV, Mende F, Wilsch-Bräuninger M, Thiele C, et al. (2004) Sterol-derived hormone(s) controls entry into diapause in *Caenorhabditis elegans* by consecutive activation of DAF-12 and DAF-16. DOI: 10.1371/journal.pbio.0020280

Keeping Proteins on Target

DOI: 10.1371/journal.pbio.0020361

To keep a cell healthy, proteins must go to the right places within a cell. To direct proteins to specific areas of the cell, they're marked with tags akin to zip codes on mail. These tags can direct one protein to the cell's nucleus, where it regulates gene expression, say, while another is sent to the hinterlands of the cell membrane, where it receives environmental signals. Protein targeting is crucial even as cells are building proteins—otherwise, for example, proteins won't fold into the proper shape.

There's a pair of proteins that, across all organisms, plays a key role in protein targeting. Called Ffh and FtsY in bacteria, these proteins each have an active and inactive state; only when they're in their active state can they bind each other and deliver other proteins to their proper location. Both these proteins are GTPases, a class of proteins that work as molecular switches. However, among GTPases, Ffh and FtsY are unique. Other GTPases switch between active and inactive states by binding different forms of a small, energy-carrying molecule—either GTP or GDP. Such GTPases often require the help of other proteins to switch states. Ffh and FtsY, on the other hand, almost always have GTP bound. And when they interact, they change each other's state—allowing each other to convert GTP to GDP—without the help of other proteins. But researchers didn't know exactly how these proteins interacted or how they switched between their active and inactive forms.

Now, as reported in this issue of *PLoS Biology,* Shu-ou Shan and colleagues have found that when Ffh and FtsY bind and then activate each other, they likely go through an unusual, multi-step process in which the proteins change shapes, flexing so that different parts of the proteins become active at each step.

The researchers mutated 45 different sites in the gene that encodes the FtsY protein to produce a bevy of mutant proteins with different properties. The researchers chose mutations that produced amino acid substitutions at sites in the FtsY protein that have been preserved through evolution, and so are presumably crucial for the protein's function. These sites, it turns out, are all on the protein's surface where it interacts with Ffh.

The mutant versions of FtsY varied in how well they bound to the normal version of Ffh and how quickly the two proteins activated each other. The researchers were able to sort the different mutations into four different classes based on the type of problem the proteins had: some bound Ffh very loosely, some bound Ffh well but only weakly turned on its GTPase activity, and so on. All of these mutations would presumably foul up the protein targeting system, so this explains why certain amino acids have been preserved through evolution.

Both Ffh and FtsY change shapes as they bind, activate each other's GTPase activity, then cleave GTP and release each other, the researchers infer. They don't have direct evidence for these shape changes, but the postulated bends and twists during interaction are consistent with the build of the proteins. These shape changes, they speculate, could switch different parts of each protein between active and inactive states. By showing how this unique type of GTPase switch likely works, Shan and colleagues have helped explain how cells target proteins to specific areas—and perhaps have paved the way for others to find similar switches elsewhere within cells.

Shan S, Stroud RM, Walter P (2004) Mechanism of association and reciprocal activation of two GTPases. DOI: 10.1371/journal.pbio.0020320

A Mechanism of Prion Propagation

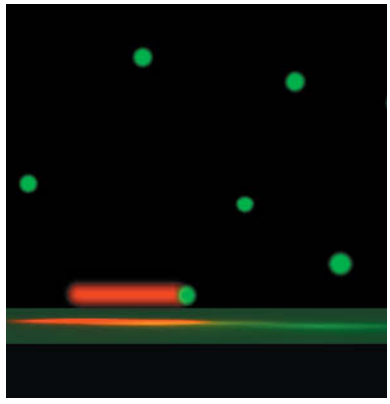
DOI: 10.1371/journal.pbio.0020360

The key to any protein's function is its structure. Proteins first emerge as a linear strip of amino acids from the cellular protein-manufacturing machinery, and it is this primary sequence that determines a protein's ultimate conformation. Improperly folded proteins—which can gum up cells and, when secreted, tissues—are normally destroyed. But in a wide range of diseases, including prion (from proteinaceous and infectious) diseases and neurodegenerative diseases like Parkinson disease and Alzheimer disease, amyloid fibrils, or plaques—misshapen proteins that aggregate into characteristic rope-like configurations—accumulate in tissue.

When amyloid precursors and prions (pronounced PREE-ons) lose their normal conformation, they acquire the ability to infect their neighbors. Like molecular dominoes, the fall of one malformed protein precipitates the downfall of its neighbors, as one protein after another assumes the misshapen form of the first. Any chance of developing methods to contain the expansionist tendencies of these proteins depends on understanding the mechanism of propagation, an area of active research.

An abundance of small protein aggregates, called oligomers, is associated with amyloid fiber growth and formation. (Single proteins are called monomers; they "polymerize" into longer chains.) Mounting evidence suggests these so-called amyloid intermediates are the "toxic species" underlying amyloid diseases. The steps in amyloid formation, however, are unclear: Must amyloids follow a progression from monomer to oligomer to plaque? That is, are oligomers required for amyloid plaque formation? Using the yeast prion protein Sup35 to study how amyloids form, Jonathan Weissman and colleagues propose a model of amyloid plaque formation and show that it can indeed occur in the absence of the putative toxic oligomers.

In yeast, the Sup35 protein forms self-replicating aggregations reminiscent of amyloid formation and prion propagation. Though yeast aren't susceptible to prion diseases, they do assume what scientists call the yeast prion state. Two protein domains called NM together form self-propagating amyloid fibers that give rise to the yeast prion state. Oligomers, which are typically seen when other proteins form amyloids, have also been seen during this process, some of them near NM



DOI: 10.1371/journal.pbio.0020360.g001

Schematic of single-molecule fluorescence experiment used to establish amyloids growth mechanism

fiber ends. Weissman's team wanted to know what these oligomers were doing.

To investigate the role of oligomers in NM amyloid formation and growth, the researchers explored the relationship between monomer concentration and polymerization progress. Initially, fiber growth rate was tied to the concentration of NM monomers; but as concentrations increased, growth rate was moderated by NM conformational changes caused after binding to the fiber ends. Shaking the samples increased polymerization rate.

During polymerization reactions, the authors observed a pronounced pause, followed by an abrupt increase in polymerization rate. Since the length of the pause showed only a weak dependence on the concentration of monomers, Weissman and colleagues explain, this finding could not be explained by a simple model of nucleation polymerization, in which growth occurs monomer by monomer, emerging from a monomer "nucleus."

Instead, Weissman and colleagues' findings support a model in which nucleated monomers initially support fiber growth, fibers undergo fragmentation, and monomers rapidly grow from the broken ends. Weissman and colleagues confirmed that the fibers were growing monomer by monomer by attaching to fragmented fiber ends with fluorescent microscopy, which can detect single molecules.

Though the authors do not rule out the possibility that oligomers could attach to the fiber ends as well, their results show that amyloid growth can occur independently of oligomers. Since many of the properties observed in Sup35 polymerization are evident in other amyloid-forming proteins, the model presented here may be shared as well. Future studies will have to explore this question, along with the issues of how oligomers figure into the process and how they cause disease. Weissman and colleagues raise the possibility that creating conditions that favor fiber growth while inhibiting oligomer formation might limit the toxic effects of amyloid plaques. The approaches outlined here should lay the foundation for exploring these questions in higher organisms.

Collins SR, Douglass A, Vale RD, Weissman JS (2004) Mechanism of prion propagation: Amyloid growth occurs by monomer addition. DOI: 10.1371/journal.pbio.0020321

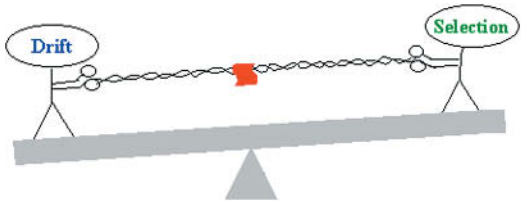
Spotting Signs of Natural Selection

DOI: 10.1371/journal.pbio.0020344

Milk, cheese, and yogurt are so ingrained in the diets of Europeans that it's easy to forget that their ancestors ever ate differently. But about 9,000 years ago, before the domestication of cows, sheep, and goats, milk was a staple only for babies. Back then—just as in most Asian and African cultures today—individuals lost their ability to digest lactose, a sugar found in milk, as they grew up.

But with the domestication of animals, milk became abundant. Among herders, individuals had an advantage if they had versions of genes, also known as alleles, that allowed them to digest lactose into adulthood. They would tend to be healthier and reproduce more than those who could not digest lactose. Thus, by natural selection within herding groups, over generations those who could drink milk into adulthood became more common.

Researchers have found the allele that allows adults to digest lactose, and it's one of the clearest signs of natural selection in humans. In groups with a history of herding, the vast majority of people have the allele, whereas in non-herding groups, most people lack it. Researchers can find such footprints of selection by comparing groups of people that have lived in different environments, for example, or have eaten different diets.



DOI: 10.1371/journal.pbio.0020344.g001

In this issue of *PLoS Biology*, Joshua Akey and colleagues report new-found signs of natural selection in several human genes—including a chunk of Chromosome 7 encompassing four genes, the largest footprint of selection found yet. The research group analyzed the complete sequences of 132 genes in a set of 23 European-Americans and 24 African-Americans. All these genes are involved in inflammation, blood clotting, or blood pressure regulation and were studied as part of a larger project looking for alleles that contribute to disease.

In general, solid evidence of natural selection acting on genes is hard to find. The history of selection can be obscured by a variety of processes. For one, genes can undergo "neutral changes," in which some of its base pairs change, but without altering the sequence or function of the protein the gene codes for. Also, idiosyncrasies in the history of a population can leave marks on the gene pool. A lineage can go through a "bottleneck," for example, if a small group splinters off from a larger population and then later multiplies. In general, the splinter group won't perfectly represent the larger population, so the frequencies of alleles for many genes will be skewed in the splinter group's lineage.

Having first ruled out irrelevant changes in genes and population history effects, Akey and colleagues found strong signs of natural selection only in the European-Americans, suggesting this group went through significant changes in climate, diet, or culture more recently than the African-American group. This fits with the well-accepted idea that European populations came from small groups that split off from the larger African population. The researchers find evidence for such an event in the European population about 40,000 years ago. They also estimate that the region of Chromosome 7 was subjected to strong selection around 10,000 years ago, roughly when European herders began drinking milk. Interestingly, two of these genes, *TRPV5* and *TRPV6*, limit the rate of calcium uptake, so selection on one or both of these genes in Europeans could have originated with herding.

Recent studies also found *TRPV6* to be more active in prostate cancer cells. In addition, African-Americans suffer higher rates of prostate cancer, and Akey and colleagues found that European-Americans have alleles of *TRPV6* different from those of African-Americans. Given this evidence, the researchers suggest that this gene may be involved in susceptibility to prostate cancer. This research could therefore shed light on the evolution of complex diseases such as cancer and why different populations suffer different rates of disease.

Akey JM, Eberle MA, Rieder MJ, Carlson CS, Shriver MD, et al. (2004) Population history and natural selection shape patterns of genetic variation in 132 genes. DOI: 10.1371/journal.pbio.0020286

Do Genes Respond to Global Warming?

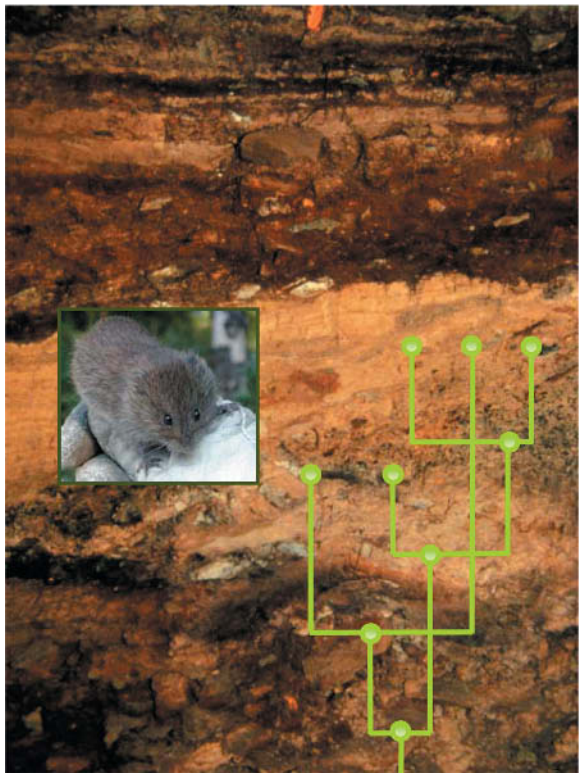
DOI: 10.1371/journal.pbio.0020338

Scientists continue to argue the extent that human activities drive global warming, but few would argue that it exists. The International Panel on Climate Change predicts that greenhouse gases will increase global temperatures by 3.6 degrees F by 2100—a rise unprecedented over the past 10,000 years. What might the world look like as we approach that point? Wetlands will disappear. Floods, hurricanes, and droughts will become progressively more severe. Infectious diseases will increase in virulence and range. Montana's famed glaciers may all but disappear within 30 years. A quarter of species may vanish by 2050.

While the effects of climate change on species' geographic ranges and population dynamics have been studied to some extent, scientists know little about how species respond to climate change at the genetic level. In this issue, Elizabeth Hadly and colleagues analyze three different dynamic processes—environmental change, population response, and gene diversity fluctuations—and present evidence that climate change influences variation in genetic diversity.

Focusing on two mammal species—the Montane vole and the northern pocket gopher—Hadly et al. asked how the two species responded to historical climate-induced habitat alterations in northwestern Wyoming. They gathered fossils from Yellowstone National Park's Lamar Cave, which contains a treasure trove of carbon-dated deposits that mirror the community of mammals in the area today. Comparing genetic material extracted from fossil samples from different time points over the past 3,000 years to genetic data taken from contemporary animals, Hadly's team tracked genetic changes in populations of the two species and used this information (along with relative fossil abundance and modern population density) to estimate changes in effective population size over time. (Effective population size refers to the number of individuals contributing genetic material to the next generation. Populations with a small effective population size, for example, would be highly vulnerable to environmental catastrophe.) The genetic and demographic data were then combined with environmental records to analyze the relationship between the factors.

Studying these populations in space and time—an approach the authors call "phylochronology"—offers an opportunity to analyze the genetic diversity of a species against the backdrop of environmental fluctuation within an evolutionary time frame. It also suggests how microevolutionary forces—factors that affect genetic variation in populations over successive generations—shape genetic responses to climate change. Such evolutionary forces include mutation, genetic drift (the random gene fluctuation in small populations that stems from the vagaries of survival and reproduction), and gene flow (changes in the gene frequency of a population caused by migration).



DOI: 10.1371/journal.pbio.0020338.g001

The past 3,000 years includes two periods marked by dramatic climate change—the Medieval Warm Period and the Little Ice Age—that had different effects on local mammal populations depending on their habitat preferences. Habitat specialists, the vole and pocket gopher live in the wet mountain regions of western North America. Though both showed population increases during wetter climates and declines during warmer periods, Hadly et al. predicted the gene diversity fluctuations of the two species would differ based on their different ecological behaviors. And that's what they found: genetic response is tied to population size. Pocket gophers have low population densities, stick close to home, and are fiercely territorial, while voles live in high-density populations and range more widely. For the gophers, population declines resulted in reduced gene diversity; for the voles—which have a larger effective population size and greater dispersal between populations—population declines resulted in increased gene diversity. But what forces underlie these differences in genetic variation?

A recent study suggests that migration (a primary agent of gene flow) is most common in and between low-density patches in vole populations, which implicates gene flow as the driver of gene diversity patterns. But the authors don't rule out selection as a possibility, and suggest how to go about resolving the question. Hadly et al. show that phylochronology opens a unique window onto the relatively recent evolutionary past and offers "the potential to separate cause from effect." They also conclude that "differences in species demography can produce differential genetic response to climate change, even when ecological response is similar." With a 3-degree temperature increase in just the past 50 years in the American West, conservation of biodiversity may well depend on such insights.

Hadly EA, Ramakrishnan U, Chan YL, van Tuinen M, O'Keefe K, et al. (2004) Genetic response to climatic change: Insights from ancient DNA and phylochronology. DOI: 10.1371/journal.pbio.0020290

Natural Biodiversity Breaks Plant Yield Barriers

DOI: 10.1371/journal.pbio.0020331

The birth of agriculture, some 10,000 years ago in the Middle East's Fertile Crescent, revolutionized human culture and society. Refined farming techniques led to increased yields and freed humans from the demands of constant foraging. Along with that freedom came social complexity, division of labor, improved standards of living, and a measure of leisure time. Agriculture also led to overpopulation followed by starvation, conflict over fertile farming land, and environmental damage. For the Maya and other civilizations, such consequences proved fatal.

Many consumer and environmental groups believe that modern industrial agricultural practices like factory farming of animals and genetic engineering of crops threaten to bring similar ruin. But with 6 billion people living on the planet—a figure that's expected to increase 50% in just 50 years—many plant scientists believe that feeding a burgeoning population will require the tools of biotechnology. Plant breeders face the daunting challenge of developing high-yielding, nutritious crops that will improve the global quality of life without harming the environment or appropriating dwindling natural habitats for agricultural production. A major roadblock to feeding the world is a continuing decline in the genetic diversity of agricultural crops, which has in turn limited their yield improvement. (Domestication often involves inbreeding, which by definition restricts the gene pool.) Now Amit Gur and Dani Zamir of Hebrew University report a way to lift these productivity barriers by tapping into the natural diversity of wild plants.

Traditional plant breeders improve the quality and yield of crops by crossing plants with desired traits to create a new, hopefully improved, hybrid strain. But traditional breeding is limited by the available gene pool of a cultivated plant species and eventually hits a wall—reshuffling the same genetic variation can boost yield only so much. With the advent of biotechnology, plant scientists were buoyed by the prospect of improving plants through genetic modification. But aside from a few successes with introducing single-gene herbicide- and pest-resistant traits, most plant traits have proved too complex to repay the incorporation of a single transgene—that is, a gene taken from a different species—with the hoped-for response. Biotech-based investigations and applications in plant science have also been hampered by consumer reaction against genetically modified organisms. (For more on the techniques of modern plant breeding, see the essay "Diversifying Selection in Plant Breeding," also in this issue.)

Faced with these limitations, Gur and Zamir tried another approach—a back-to-nature approach. "Natural biodiversity is an unexploited sustainable resource that can enrich the genetic basis of cultivated plants," they explain in the report. The distantly related wild cousins of cultivated plants can be seen as a "huge natural mutagenesis resource" with novel gene variants that can increase productivity, quality, and adaptability. Not only that, the genetic material of wild plants—every gene and regulatory element—has already been refined and tested by over a billion years of evolution and natural selection.

To identify genomic regions in wild tomato species that affect yield, Gur and Zamir created a population of hybrid crosses of a wild tomato species and a cultivated tomato species; each line had a single genomic region from the wild tomato inserted into the cultivated plant. Rather than introducing a single wild tomato gene into the cultivated plants, the authors used a "pyramided" strategy that combined three independent yield-enhancing genomic regions from the wild species into the new plant line. Plants were grown over three seasons, during which they were exposed to different environments, including drought. By combining traditional phenotyping techniques—which characterize the plant's physical traits based on its genetic makeup—with genetic marker analysis, the authors identified a number of wild tomato genomic regions that increased yield.

Their results demonstrate that an approach based on biodiversity—which takes advantage of the rich genetic variation inherent in wild relatives of cultivated crops—can produce varieties that outperform a commercially available hybrid tomato in both yield and drought resistance. Gur and Zamir attribute the improved performance to their unique pyramiding strategy. Their hybrid model—applying the tools of modern genomics to traditional plant breeding—offers plant breeders a powerful approach to improving the quality and yield of cultivated plants by taking advantage of the inherent biodiversity of the natural world. It's a strategy that may well apply to rice, wheat, and other vital staples of the world's food supply.

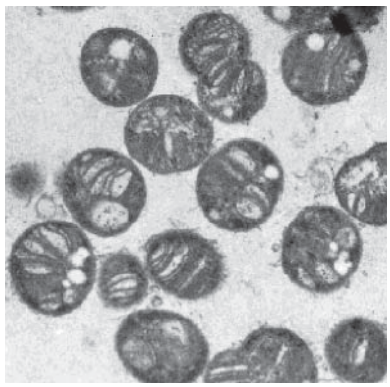
Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. DOI: 10.1371/journal.pbio.0020245

The Genome of a Methane-Loving Bacterium

DOI: 10.1371/journal.pbio.0020358

Mention greenhouse gases to most people and they're apt to think of carbon dioxide, fossil fuels, and big cars. Though carbon dioxide emissions are the major source of greenhouse gases, methane is far more effective at trapping heat in the atmosphere. Like increasing carbon dioxide levels, rising levels of atmospheric methane have been attributed to human activity, mostly in the form of landfills, natural gas and oil processing (about 60%), domesticated livestock (cattle account for about 75% of livestock contributions), and rice fields (up to 29% of total emissions).

Ruminants—from cows and water buffalo to llamas and vicunas—emit methane gas as a natural by-product of their digestive process, which confers a unique ability to digest cellulose. Ruminants don't digest cellulose directly, but depend on a variety of microbes living in their rumen (main stomach) to do it for them. These microbes ferment cellulose, breaking it down into products the ruminant can digest. During this process, some microbes—bacteria called methanogens—produce methane, which ruminants expel by eructation (otherwise known as belching).



DOI: 10.1371/journal.pbio.0020358.g001

Methylococcus capsulatus **cultured in the presence of a high concentration of copper (Image: Anne Fjellbirkeland)**

Luckily, there are microbes, called methanotrophs, that consume methane. A

type of aerobic bacteria, methanotrophs oxidize methane as an energy and carbon source using the enzyme methane monooxygenase. They've been found in soils, landfills, sediments, hotsprings, and peat bogs, among other environments. Methanotrophs have been the subject of increasing interest because they can use methane as a sole source of carbon and energy—which means they play a major role in global carbon cycles—and could dramatically reduce biologically generated methane emissions. They've also been the focus of bioremediation efforts aimed at environmental decontamination. And now, with the publication of the first complete genome sequence of a methanotroph, such efforts will be all the easier. In this issue of *PLoS Biology,* a multidisciplinary team spanning the fields of genomics, bioinformatics, microbiology, evolutionary biology, and molecular biology report the complete genome sequence of *Methylococcus capsulatus* and shed light on the metabolism and biology of this ubiquitous microbe.

Contained in a single, circular molecule, the *M. capsulatus* genome comprises about 3.3 million base pairs—which is about average for a free-living bacterium—with an estimated 3,120 genes. The genome appears well-equipped to meet the specialized needs of this methanotroph, with what appear to be multiple pathways involved in the metabolism of methane and duplications of genes that code for methane monooxygenases, which are essential for the first step of methane oxidation.

Ward et al. also found evidence of "genomic redundancy" in methane oxidation pathways, suggesting that *M. capsulatus* employs different pathways depending on the availability of molecules needed to sustain cellular activities. Most surprising, the researchers note, was evidence that this methane specialist can turn into a sort of metabolic generalist—with a capacity to use sugars, hydrogen, and sulfur—and appears able to survive reduced oxygen levels. These genome-based hypotheses will require experimental validation, the authors note, but could have important implications for *M. capsulatus* ecology—including what environments might be amenable to methanotroph-mediated bioremediation.

The genomes of important microbial players in the carbon cycle—including microbes involved in photosynthesis and methanogenesis (methane production)—have already been sequenced. With the addition of a sequenced methanotroph genome, scientists can systematically investigate different genes and regulatory elements to better understand how these methane consumers fit into this global cycle. The *M. capsulatus* genome provides a platform for investigating the details of methanotroph biology and its potential as a biotech workhorse. It may also guide efforts to harness this bacterium's penchant for methane to reduce global greenhouse gas emissions, to degrade chlorinated hydrocarbons and other persistent pollutants, and to produce protein for animal feed.

Ward N, Larsen Ø, Sakwa J, Bruseth L, Khouri H, et al. (2004) Genomic insights into methanotrophy: The complete genome sequence of *Methylococcus capsulatus* (Bath). DOI: 10.1371/journal.pbio.0020303

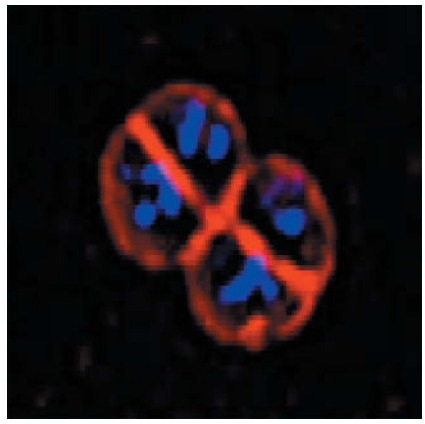
Damage Response Protein Buys Time for Bacterial DNA Repair

DOI: 10.1371/journal.pbio.0020325

It is often said that after a nuclear catastrophe, cockroaches will inherit the earth, because they are so resistant to the harmful effects of ionizing radiation. But should the unthinkable come to pass, the bacterium *Deinococcus radiodurans* is likely to outlast even the cockroach. Its ability to endure radiation is truly impressive: it can withstand a dose a thousand times that which will kill a human. How it accomplishes this phenomenal feat of survival is the subject of a study in this issue by John Battista and colleagues at Louisiana State University in Baton Rouge and at the University of Wisconsin at Madison.

While radiation damages many cellular components, it is the fracturing of the cell's DNA that is the most harmful. DNA breaks can be repaired, but in doing so, the cell is racing against time. The exposed free ends of the DNA invite digestion by the cell's own enzymes, called exonucleases. If the DNA is not stitched back together quickly enough, the exonucleases will degrade it past the point of repair, and the cell will ultimately succumb. Large doses of radiation can fracture a chromosome in thousands of places, far in excess of the repair ability of most cells. *D. radiodurans*, however, largely prevents exonuclease digestion, an ability which has previously been shown to be linked to the activity of a gene with the rather uninformative name of DR0423. But how, exactly, does this gene accomplish this life-saving feat?

To answer this question, Battista and colleagues first showed that, following radiation exposure, DR0423 was upregulated 20- to 30-fold, and that deletion of the gene renders *D. radiodurans* susceptible to ionizing radiation. Together, these results clearly indicate that the DR0423 gene product is critical for protecting the bacterium. Based on this, they dubbed the gene *ddrA*, for "DNA damage response." They also found that DdrA, the protein encoded by *ddrA*, binds to single-stranded fragments of DNA, exactly like those found at the broken ends of the DNA double helix when damaged by radiation. Finally, they showed that when DdrA bound to these broken ends, they were protected from digestion by exonucleases.



DOI: 10.1371/journal.pbio.0020325.g001

***D. radiodurans* R1**

An important question about this system is what it is actually good for. Since the level of radiation tolerated by the bacterium is found nowhere on earth, of what use is such an efficient DNA protection system? The answer might be that it also protects *D. radiodurans* from the effects of desiccation, a condition much more common in the life of a bacterium, and one which also induces widespread DNA damage. While *ddrA* cannot prevent the damage, it can preserve the DNA from degradation until conditions once again allow the bacterium to function, and repair its DNA.

Harris DR, Tanaka M, Saveliev SV, Jolivet E, Earl AM, et al. (2004) Preserving genome integrity: The DdrA protein of *Deinococcus radiodurans* R1. DOI: 10.1371/journal.pbio.0020304

A Molecular Model of Blood Cell Renewal

DOI: 10.1371/journal.pbio.0020349

A developing organism captured on time-lapse video is a wonder to behold. If you're watching a chick embryo, by day 3, you'll see millions of cells engaged in a frenzy of activity, as rapidly dividing cells migrate to new positions, acquire the characteristics of specialized cells, and craft well-defined tissues, organs, and limbs in just under two weeks. In addition to the cells destined for specialization is another important group, stem cells, whose progeny have two very different fates. They can either "self renew"—that is, make identical copies of themselves—or generate intermediate progenitor cells that give rise to mature, differentiated cells.

Both differentiation and self renewal are guided by an elaborately regulated genetic program, which transforms embryonic stem cells into the many different cell types that make up the body. Adult stem cells share the hallmark trait of self renewal, but are relatively rare: in bone marrow, the source of hematopoietic, or blood-forming, only an estimated one in 10,000–15,000 cells is an adult hematopoietic stem cell (HSC).

Studies that have compared the gene expression profiles of different types of stem cells to identify genetic signatures of "stemness" have found only a limited number of signature genes. And the molecular mechanisms that regulate this so-called potency and the self renewal process have remained obscure. Now, focusing on HSCs, Margaret Goodell and colleagues have undertaken a systematic evaluation of HSC renewal. The study identifies molecular signatures associated with discrete stages of the HSC self renewal cycle and proposes a molecular model of the process.

HSC renewal passes through three stages: quiescence, activation and proliferation, and a return to the dormant state. HSCs give rise to both red blood cells, which carry oxygen and carbon dioxide, and white blood cells, which fight infection. Certain stressors—including blood-cell-inhibiting chemotherapy and bone marrow transplants—trigger HSC activation, which induces rapid proliferation, generating both progenitors to deal with the threat and new stem cells that return to quiescence. Once activated by a trigger, dormant HSCs engage a regulatory program that rapidly churns out billions of cells, then puts the brakes on cell division, prompting the return to a nondividing, quiescent state.

To understand the genetic programs underlying this process, Goodell and colleagues induced proliferation in HSCs (with the chemotherapeutic drug, 5-fluorouracil, or 5FU), then allowed the cells to return to quiescence, so they could characterize the changes in gene expression that occurred during each stage. They compared these time-specific patterns to the gene expression profiles of naturally proliferating fetal mouse HSCs (which undergo massive proliferation) and quiescent adult mouse HSCs (which hardly divide at all) to find genes associated with the two different states.

Genes were grouped into proliferating or quiescent groups based on when they were expressed after 5FU treatment, and these groupings were refined based on comparisons to previously published HSC gene expression data. Functional analysis of these genes found a bias toward genes involved in cell division processes in the proliferation stage and toward cell division inhibitors in the quiescent stage, supporting the logic of the groupings. To understand the activation process at a global level, the authors employed some novel analysis strategies, including the "Gene Ontology" (GO) system for classifying genes.

With these results, Goodell and colleagues constructed a model of the HSC self renewal cycle: quiescent HSCs maintain a "state of readiness," molecularly speaking, that allows a quick response to environmental triggers. A stressor (like the chemotherapy mentioned above) triggers a "prepare to proliferate" state—a kind of pregnant pause—and then the proliferation machinery kicks in, going through an early and late phase before quiescence returns. By shedding light on the molecular mechanisms of stem cell renewal, this study will aid efforts to develop stem-cell-based clinical therapies, which depend on replicating the HSC self renewal cycle to replenish diseased or damaged tissue, and will ultimately guide efforts to grow stem cell colonies outside the body, a long-standing goal that would have many clinical applications. The authors suggest their findings may also be relevant to studies of cancer stem cells, tumor cells with self renewal properties.

Venezia TA, Merchant AA, Ramos CA, Whitehouse NL, Young AS, et al. (2004) Molecular signatures of proliferation and quiescence in hematopoietic stem cells. DOI: 10.1371/journal.pbio.0020301

We Move in Mysterious Ways

DOI: 10.1371/journal.pbio.0020370

A man in a suit and bowler hat walks awkwardly down the street, each convoluted step a labored movement. He lifts up one knee, then briefly stoops. Stepping forward, he swings the other leg out to the side then kicks high in the air. In this old Monty Python skit, the man works for the Ministry of Silly Walks. It's his job to walk this way. The rest of us, however, tend to stroll along—or throw baseballs, or lift coffee mugs—in a much more efficient manner.

There's a nearly infinite number of silly walks, throws, and lifts, but somehow people tend to settle on one best way of doing these things. However, scientists studying motor control have been hard pressed to figure out what exactly we're doing when we move. People may be striking a balance between sloth and speed: too slow and our throws lack oomph; too fast, and instead of dunking our donuts in our coffee, we dunk our whole fist. Or people might be minimizing some version of jerk—physicists' and engineers' term for changes in acceleration. (Roller coaster engineers, for example, balance jerk against speed and *g*'s to keep the ride smooth and safe, but also fun.) But so far, such models that start by assuming people minimize error or jerk haven't allowed researchers to deduce what dictates how people move.

To help solve this recalcitrant problem, Konrad Körding and colleagues, as reported in *PLoS Biology,* took a page from economists, who have long used equations called utility functions that incorporate the costs and benefits of a situation. Say you like oranges better than apples, but oranges cost more. Given a certain budget for fruit, the utility function says how many of each you should buy. Similarly, Körding and colleagues observed people's preferred movements, then inferred an underlying utility function that presumably describes bias in the nervous system for different movements.

To see which movements people preferred, the researchers engaged people in a simple virtual reality system. The subjects moved a joystick that fought back: it was connected to a set of motors that produced varying forces—with a strong force for a short time, say, or a mild

force for a longer time. Over and over, the subjects moved their cursors from one spot to another. After each pair of moves, the subjects then chose which of the two movements they found easier.

In this way, the researchers were able to rank a large set of different movements relative to each other by individuals' preferences. They found a surprising amount of agreement among the subjects on which movements were preferable. They also got a counterintuitive result: as the duration of the resistance got longer, people actually preferred stronger resistance. The researchers speculate that subjects didn't mind larger resistance when it acted over a longer period because the force takes longer to ramp up to its maximum value. Subjects would have more time to adjust—just as when someone gradually pushes into you, you can stay standing by leaning into them, whereas if they shove you with the same force it can knock you off balance.

By showing that utility functions can be of use not only in explaining the marketplace but also motor control, Körding and colleagues have added a new tool to biologists' repertoire. Though their approach hasn't closed the case on the mysteries of movement, it could help explain why we settle for a particular, non-silly walk.

Körding KP, Fukunaga I, Howard I, Ingram J, Wolpert DM (2004) A neuroeconomics approach to inferring utility functions in sensorimotor control. DOI: 10.1371/journal.pbio.0020330

A Test Case for DNA Barcodes to Identify Species

DOI: 10.1371/journal.pbio.0020357

One hundred years before Darwin returned from his voyage on the *H. M. S. Beagle* "struck with certain facts" that "seemed to throw some light on the origin of species," Linnaeus published the first systematic taxonomy of life. In *Systema Naturae,* the Swedish botanist divided organisms into plants, animals, and minerals, eventually assigning scientific names to 7,700 plant and 4,400 animal species, and popularizing the binomial system—as in *Homo sapiens*—of naming species.

In the 1700s and 1800s, naturalists classified organisms based on morphology, devoting their careers to naming newfound plants and animals. Today biologists still use Linnaean taxonomy as the foundation of scientific classification. But with just a fraction of the estimated 5–30 million species on the planet already named and too few specialists to do the job, biologists are looking for high-throughput tools that can rapidly and accurately identify both individuals of a species and entirely new species. That's what some scientists say the DNA barcode will do. The DNA barcode, as the name implies, uses genes to identify species much like supermarket barcodes identify products. The idea is that a short stretch of genetic code from a reference gene is unique enough to one species to distinguish it from every other species, and that comparisons of sequence variations in that stretch of gene can reveal evolutionary relationships among species.

Such technology could radically advance biologists' attempts to achieve the long-standing goal of cataloging life on earth, but the approach is controversial, with critics questioning both the method and its applications. (For more on the debate, see "DNA Barcoding: Promise and Pitfalls," also in this issue.) Paul Hebert and colleagues offer a proof of the utility of the DNA barcoding concept, using a 648-basepair region of a mitochondrial gene (cytochrome *c* oxidase I, or COI) in a study of 260 North American bird species.

Mitochondria—the cell's power generators—contain their own DNA, and mitochondrial DNA (mtDNA) evolves much faster than nuclear DNA. It evolves so quickly, in fact, that mtDNA sequence variation has been found not just between closely related, or sister, species but also within species. Still, the variation is much greater among than within species, which is why mtDNA divergences have become a tool for identifying species.

Hebert and colleagues tested the effectiveness of the mtDNA COI barcode by matching bird species flagged by the COI barcode against those already established by taxonomic methods. The litmus test for DNA barcoding is absence of COI sequence overlap between species. Beyond that, differences within species should be significantly fewer than those between species. And that's what the researchers found. All 260 species had unique COI barcodes, with differences between species for the most part much more frequent—on average, 18 times more common—than those within species. In the 130 species represented by two or more individuals, COI sequences were either identical or closest to other sequences within that species. For these 260 bird species (of the 667 bird species that breed in North America), the authors report, the COI barcodes "separate individuals into the categories that taxonomists call species."

The COI barcode, the authors propose, could help resolve problematic classifications based on morphology, as arise when populations of a single species acquire distinct characteristics after geographic barriers prevent their interbreeding. For example, the similar COI sequences found in American and black oystercatchers here support taxonomic studies suggesting that they are actually color morphs of one species. And conversely, highly divergent COI sequences might bolster taxonomic studies indicating that lineages of uncertain status are indeed distinct species.

Future studies will have to determine whether these results can be generalized to animals in other climes and ecosystems, but the authors argue that constructing a comprehensive library of barcodes will facilitate such efforts. Hebert and colleagues conclude that the success of DNA barcoding depends not only on such a repository—with sequences pegged to well-characterized species exemplars—but also on the expertise of trained taxonomists. The hope is that large-scale, standardized testing based on a uniform barcode sequence could go a long way toward finishing what Linnaeus started: a full classification of all plant and animal life. To E. O. Wilson, every species is "a masterpiece of evolution, offering a vast source of useful scientific knowledge because it is so thoroughly adapted to the environment in which it lives." Faced with what Wilson calls the "worst wave of extinction since the dinosaurs died," the need for a fast and easy way to identify species has never been greater.

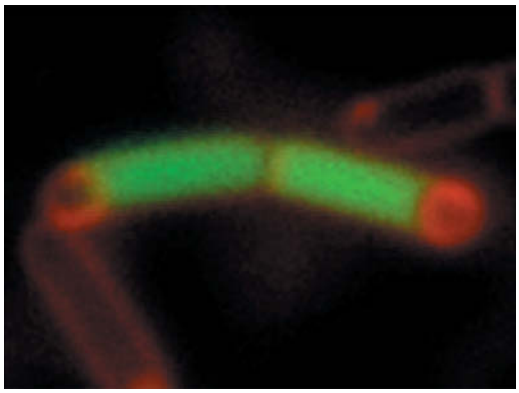
Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. DOI: 10.1371/journal.pbio.0020312

How to Make a Mother in Five Easy Steps

DOI: 10.1371/journal.pbio.0020359

Assembling a complex structure like an automobile requires the tight coordination of hundreds of independent entities—parts must be shipped and arrive on time, workers with the right skills must be in the right place on the assembly line, four (not three, not five) wheels must be bolted into place just so. Overseeing the entire operation is a cadre of managers, whose ability to monitor and respond to changing conditions keeps the entire process moving forward on time and in step.

A living cell is orders of magnitude more complex, and yet has no omniscient manager at the helm. So how does a cell make anything happen on time, and equally important, keep everything from happening all at once? These central questions in developmental biology now have the outlines of an answer in one species, *Bacillus subtilis.* In this issue, Richard Losick and colleagues show that spore formation in this bacterium is ultimately governed by the temporal interactions of five genes, which together coordinate the activity of almost 400 others.



DOI: 10.1371/journal.pbio.0020359.g001

A pair of *Bacillus subtilis sporangia,* consisting of a large mother cell (green) and a forespore (red)

When conditions are right, *B. subtilis* divides to form two different cell types: one is a resistant spore, and the other is a mother cell, which engulfs the spore and surrounds it with a protective coat. Building on the large literature addressing the genetic events underlying mother-cell development, Losick et al. performed a variety of experiments to determine exactly which genes turned on and off when, and which genes controlled which others. Over the five-hour process of mother-cell development, they determined that 383 individual genes were activated, representing 9% of the bacterium's 4,106 genes.

The instigator of the entire process is a protein called sigma-E (σ^E). Sigma factors, such as the sigma-E protein, bind to RNA polymerase, and in so doing, increase its affinity for, and therefore its ability to activate, other genes. Thus, sigma factors preferentially activate a specific set of genes. Sigma-E turns on 262 genes (which together compose its "regulon"), kick-starting a variety of processes in the early development of the mother cell.

Importantly, two of its targets, SpoIIID and GerR, are genes for DNA-binding proteins, which themselves modulate the expression of genes in the middle phase of development. Part of SpoIIID's portfolio is turning off transcription of a portion of the sigma-E regulon, and amplifying transcription of another portion. This type of control circuit, in which A leads to B, and then both A and B influence C, is called a feed-forward loop.

Among the joint targets for sigma-E and SpoIIID is another sigma factor, sigma-K (σ^K). By generating the DNA-binding protein GerE, this factor begins a second feed-forward loop, and together, sigma-K and GerE activate the final set of genes needed for mother-cell development. In outline, the system looks like this: sigma-E → SpoIIID/GerR → sigma-K → GerE.

The consequence of all this activity is a series of transcriptional pulses, timed to supply proteins just as they are needed, and then turn off their production when the need passes. For instance, to form the multilayered coat around the spore, sigma-E turns on genes that form the bottom layer, or substratum; these are turned off by SpoIIID. Genes for outer layers, also turned on by sigma-E, are not turned off by SpoIIID, but instead by GerE. Sigma-K turns on genes which form the polysaccharide surface of the coat, which is needed later on.

The elucidation of this complex pattern of gene expression doesn't by any means answer every question about *B. subtilis* development, let alone development in more complex organisms. There is much still to be learned about how genes lower down in the hierarchy—the "middle managers"—do their jobs, and how the system is fine-tuned by environmental conditions. And while the general scheme of feed-forward loops and hierarchical control is likely to apply to multicellular, eukaryotic organisms, the details are certain to be different, and much more complex.

Eichenberger P, Fujita M, Jensen ST, Conlon EM, Rudner DZ, et al. (2004) The program of gene transcription for a single differentiating cell type during sporulation in *Bacillus subtilis*. DOI: 10.1371/journal.pbio.0020328