

Synopsis of Research Articles

Tubeworm May Live Longer by Cycling Its Sulfur Downward

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Any organism may be limited by some essential nutrient in short supply—nitrogen for a plant on poor soil, for instance, or iron for phytoplankton in the open ocean. For the long-lived tubeworm *Lamellibrachia luymesii*, what it needs most and has least is sulfide. *L. luymesii* lives clustered around hydrocarbon-releasing ocean floor seeps in the Gulf of Mexico, and it—or rather, its menagerie of internal bacterial symbionts—uses the high-energy sulfide the way plants use sunlight, extracting energy and releasing the waste products, in this case sulfate.

With a lifespan of up to 250 years, *L. luymesii* is among the longest-lived of all animals, but how it obtains sufficient sulfide to keep going for this long has been a mystery. In this issue, Erik Cordes and colleagues propose a model in which, by releasing its waste sulfate not up into the ocean but down into the sediments, *L. luymesii* stimulates the growth of sulfide-producing microbes, and ensures its own long-term survival.

The sulfide *L. luymesii* needs is created by a consortium of bacteria and archaea that live in the sediments surrounding the vent. These chemoautotrophs use energy from hydrocarbons to reduce sulfate to sulfide, which *L. luymesii* absorbs through its unique “roots,” extensions of its body that it tunnels into the sediments. Measurements of sulfide and sulfate fluxes in the water near the vents are inconsistent with the observed tubeworm colony size and individual longevity, leading Cordes et al. to propose that *L. luymesii* also uses its roots to release sulfate back to the microbial consortia from which it draws its sulfide.

Without this return of sulfate, the model predicts an average lifespan of only 39 years in a colony of 1,000 individuals; with it,

survival increases to over 250 years, matching the longevity of actual living tubeworms. The model, which was based largely on empirical data, is relatively unperturbed by changes in hydrocarbon seep rate, or in the growth and recruitment rates for the colony. The authors note that the proposed return of sulfate into deep sediments would, in theory, increase the local rate of carbonate rock formation, creating a barrier to fluid circulation into the sediments. Their model predicts this to occur after about 50 years, in line with observed reductions in tubeworm recruitment in colonies of this age. They propose that carbonate precipitation may be inhibited if roots can also release hydrogen ions, a possibility open to further testing. Their model also explains several biogeochemical anomalies observed near tubeworm colonies, including elevated levels of highly degraded hydrocarbons and higher than predicted rates of sulfur cycling.

To date, the proposed return of sulfate to the sediments through the roots is only a hypothesis—albeit one with much to support it—that still awaits direct confirmation. By providing a model in which this hypothetical interaction provides real benefits and explains real observations, the authors hope to stimulate further research into the biology of *L. luymesii*. For more on tubeworms, see “Microfauna–Macrofauna Interaction in the Seafloor: Lessons from the Tubeworm” (DOI: 10.1371/journal.pbio.0030102), also in this issue.

Cordes EE, Arthur MA, Shea K, Arvidson RS, Fisher CR (2005) Modeling the mutualistic interactions between tubeworms and microbial consortia. DOI: 10.1371/journal.pbio.0030077



DOI: 10.1371/journal.pbio.0030108.g001

An aggregation of *Lamellibrachia luymesii* in the Gulf of Mexico (Photo: Ian MacDonald)

Fish Need You

DOI: 10.1371/journal.pbio.0030075

There is little in biology that compares in beauty and limpidity to the development of a zebrafish embryo as viewed through a light microscope. The transparent eggshell and embryo tissues expose the minutest details of cell migrations and organ assembly to the curious viewer. Within a day, distinct vertebrate features emerge: a distinct head with the outlines of two large eyes, a quickly pumping heart, a notochord,

and a growing array of somites—the bone and muscle precursors—stretching from trunk into tapering tail.

The transparent zebrafish embryo has allowed geneticists to discover a large number of mutants with anomalies in the development of external and internal organs. Seven mutations, collectively known as “You-class,” turn the pointed, chevron-like somites into shallow, rounded arcs (“You” stands for “U-

shaped”). Ian Woods and William Talbot now show that the You mutation disrupts a new modulator of Hedgehog signaling.

Hedgehog is an extracellular signaling protein that can impose various fates on target cells at close proximity or over longer distances. Much research is focused on understanding the factors that promote or limit Hedgehog’s activity and range. Woods and Talbot propose that the You protein acts in the

extracellular environment to promote Hedgehog signaling.

Hedgehog was originally named for mutations that cause excess brush-like denticles to grow on the surface of fruitfly embryos, but it is now known to direct countless developmental decisions in invertebrates and vertebrates alike. In addition, several cancers are known to result from inappropriate Hedgehog signaling. In fish, Hedgehog's best-documented role is in muscle development. In the absence of Hedgehog signaling, cells destined to become slow muscle fibers fail to differentiate properly. A subset of these slow muscle cells—the muscle pioneers—congregate near the dorso-ventral midline of the embryo, where the dorsal and ventral halves of somites converge. When these specialized cells are absent, abnormal somite assembly leads to the U-shaped phenotype.

The authors found that You mutants showed many telltale signs of reduced Hedgehog signaling. Proteins that are normally expressed at certain times during the development of slow muscle

cells were not activated in You mutants, indicating that these cells did not form. Mutant embryos also displayed reduced expression of the Hedgehog receptor Patched, a universal reporter of Hedgehog signaling activity. In addition, You mutants had specific ventral spinal chord defects that are shared by known Hedgehog pathway mutants. Yet You mutants expressed Hedgehog normally. Moreover, Hedgehog targets could still be activated in You mutants in response to excess Hedgehog signaling, suggesting that the signaling cascade is left intact. The authors concluded that the You protein was a facilitator rather than a crucial transmitter in Hedgehog signaling, likely acting at a step upstream of a cell's response to Hedgehog. Normal muscle pioneers could form in chimeric embryos (embryos made of wild-type and You mutant cells) regardless of which cells—the Hedgehog-producing cells or Hedgehog-responding muscle precursors—expressed You. This made it most likely that the You protein acted outside the cells, perhaps as a cell matrix component.

The authors mapped the You mutation and found that it disrupted the coding region of a gene encoding a putative secreted protein. The predicted You protein is closely related to members of the mouse SCUBE family, a group of proteins that are defined by characteristic extracellular motifs (although these proteins have not yet been linked to Hedgehog signaling). This observation strengthens the hypothesis that the You protein has extracellular functions, and the researchers' experimental evidence supports a role for You in transport or stabilization of Hedgehog. At later stages You could also participate in other signaling pathways, as its expression does not always coincide with that of Hedgehog during zebrafish development.

Woods IG, Talbot WS (2005) The *you* gene encodes an EGF-CUB protein essential for hedgehog signaling in zebrafish. DOI: 10.1371/journal.pbio.0030066

Predicting the Future: Mirror Neurons Reflect the Intentions of Others

DOI: 10.1371/journal.pbio.0030109

One of the more intriguing recent discoveries in brain science is the existence of “mirror neurons,” a set of neurons in the premotor area of the brain that are activated not only when performing an action oneself, but also while observing someone else perform that action. It is believed mirror neurons increase an individual's ability to understand the behaviors of others, an important skill in social species such as humans. A critical aspect of understanding the behavior of another person is recognizing the intent of his actions—is he coming to praise me or to bury me? In this issue, Marco Iacoboni and colleagues use functional magnetic resonance imaging (fMRI) to show that the mirror neuron system tracks not only the actions, but also the intentions, of others.

The researchers presented subjects with one of three types of movie clips, “context,” “action,” and “intention.” The “context” clip came in two versions. In the first, a mug of tea, a teapot, a pitcher of cream, and a plate of cookies sit neatly on a nondescript surface. In the second, the mug is empty, the pitcher is on its side, and a napkin lies crumpled beside scattered cookie crumbs—the dregs of an apparently well-enjoyed snack. The “action” clip shows only an empty mug, being grasped either by the whole hand around the rim (called whole-hand prehension), or with the fingers on the handle (precision grip). The “intention” clip puts it all together, providing the context needed to understand the intent of the action. In the first context scene, the mug is grasped in the context of a well-laid spread, signaling intent to drink the tea. In the second, the mug is grasped against the backdrop of the rest of the tea-time dregs, signaling the intent to clean up. In

both cases, the type of grasp used is alternated, to avoid implying intent merely based on grip type.

The essential interpretive technique in fMRI is to subtract the activation patterns from two different stimuli, thereby highlighting brain regions that are activated differentially in response to the difference in stimulus. In this study, “intention minus context” showed areas involved in recognizing both action and intention, while “intention minus action” showed areas for both context and intention. Comparing these two results, Iacoboni and colleagues found that an area in the right inferior frontal cortex, an area known to be involved in the mirror system, was activated only by scenes in which intention could be inferred. In the authors' words, “it appears that there are sets of neurons in human inferior frontal cortex that specifically code the ‘why’ of the action and respond differently to different intentions.”

They note that these neurons differ in function from previously defined mirror neurons in that they apparently code not current actions, but some aspect of future ones. In this interpretation, an action observed within a familiar context activates mirror neurons for “logically related” actions, those that most likely will follow the observed one. This suggests the mirror neuron system is intimately involved not only with understanding the behavior of others, but predicting it as well.

Iacoboni M, Molnar-Szakacs I, Gallese V, Buccino G, Mazziotta JC, et al. (2005) Grasping the intentions of others with one's own mirror neuron system. DOI: 10.1371/journal.pbio.0030079

The Few, the Strong: Rat Cortex Features Small Numbers of Powerful Connections

DOI: 10.1371/journal.pbio.0030111

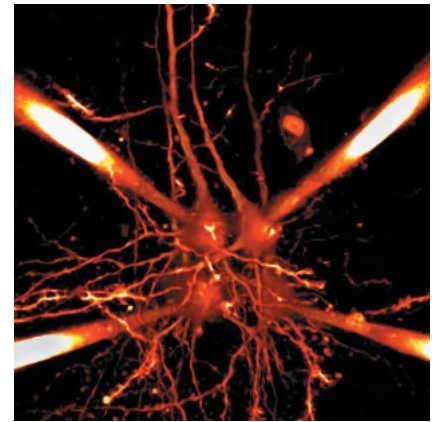
How is the brain wired up? Each neuron may connect with hundreds or even thousands of others, and the human brain has a hundred billion neurons. Determining the connection diagram for a whole brain is a truly daunting prospect, and currently well beyond reach. But one way into this thicket is to look for patterns in a small region. In this issue, Dmitri Chklovskii and colleagues show that in the rat visual cortex, some kinds of connection patterns are much more common and much stronger than chance would predict.

To determine the pattern of connections, the researchers placed electrodes into randomly chosen quartets of neurons near each other. They stimulated each in turn, and determined which members responded, and how strongly. Sampling over 800 such quartets, they found 931 actual connections out of a possible 8,050, for an average rate of connectivity of 11.6%. From the group of connected neurons, they then asked about reciprocal connections: what was the likelihood that, if A stimulated B, B stimulated A as well? They found that bidirectionally connected cells were four times as common as expected by chance, a pattern previously observed in other regions of cortex. They asked the same question for groups of three cells, for which there are 16 possible connection patterns. Two patterns stood out as especially significant: (1) A and B talk back and forth with each other, and

both listen to C; and (2) A, B, and C all talk with one another. For four cells, although the numbers were too small for statistical analysis, a common over-represented class was chain connections, a kind of a path connecting all four cells that can be drawn without lifting the pencil from the page.

Because the strength with which one neuron stimulates another can be just as important to network function as whether a connection exists at all, the authors examined connection strength as well. They found that connection strengths are distributed broadly, with some connections ten times stronger than the average connection and the strongest 17% of connections contributing half of total synaptic strength. They found that, on average, connections that were part of bidirectional pairs were about 50% stronger than unidirectional ones, and because of this, despite being fewer in number, they disproportionately contributed to the total amount of excitation in the neural network. A similar pattern was found for neuronal triplets—the most highly connected groups of neurons had the strongest connections among them.

Taken together, these results show that neural networks, at least in this portion of the rat brain, are characterized by a vocal minority of unexpectedly strong and reliable connections amidst a large number of weak ones, which suggests the strong ones may play more central roles in local computation or communication.



DOI: 10.1371/journal.pbio.0030111.g001

Recording multiple neurons simultaneously

This stands in strong contrast to the usual starting assumption of neural modelers, that connectivity is random. The exact pattern of connectivity seen here for excitatory neurons in one cortical layer (layer 5) may not be universal, and indeed, different patterns have been described in the cerebellum. Nonetheless, the essential feature seen here—a skeleton of stronger connections in a sea of weaker ones,—as the authors put it—may be an important and common functional feature of brain wiring.

Song S, Sjöström PJ, Reigl M, Nelson S, Chklovskii DB (2005) Highly nonrandom features of synaptic connectivity in local cortical circuits. DOI: 10.1371/journal.pbio.0030068

Seeds of Destruction: Predicting How microRNAs Choose Their Target

DOI: 10.1371/journal.pbio.0030114

Compare the gene number of fruitfly (13,000) to human (20,000), and it's pretty clear that complexity emerges not just from gene number but from how those genes are regulated. In recent years, it's become increasingly clear that one class of molecules, called microRNAs (miRNAs), exert significant regulatory control over gene expression in most plant and animal species. A mere 22 nucleotides long, miRNAs control a cell's protein composition by preventing the translation of protein-coding messenger RNAs (mRNAs). When a miRNA pairs with an mRNA, through complementary base pairing between the molecules, the mRNA is either destroyed or is not translated.

Hundreds of miRNAs have been found in animals, but functions for just a few

have been identified, mostly through genetic studies. Many more functions could be assigned if miRNA targets could be predicted. This approach has worked in plants, because miRNAs and their targets pair through the near perfect complementarity of their base pairs. But the molecules follow different rules in animals—duplexes contain just short stretches of complementary sequence interrupted by gaps and mismatches—which makes predicting miRNA targets a challenge.

In a new study, Stephen Cohen and his colleagues at the European Molecular Biological Laboratory in Germany establish basic ground rules for miRNA–mRNA pairing using a combination of genetics and computational analyses, and identify

different classes of miRNA targets with distinct functional properties. Although the miRNA is only 22 nucleotides long, its 5' and 3' ends seem to have distinct roles in binding. Cohen and colleagues show that miRNA functional targets can be divided into two broad categories: those that depend primarily on pairing to the miRNA's 5' end (called 5' dominant sites), with varying degrees of 3' pairing, and those that also need the miRNA's 3' end (called 3' compensatory sites). Surprisingly, miRNAs can regulate their targets simply by strong pairing with so-called seed sites that consist of just seven or eight bases complementary to the miRNA 5' end. Target sites with weaker 5' complementarity need supplemental pairing with the miRNA's 3' end to function. The finding that so

little sequence complementarity is needed means that there are many more target sites than had been previously recognized.

The miRNA 3' end, while not essential, is expected to confer some function, since it tends to be conserved in animals—miRNA 3' ends provide an additional measure of regulatory control by permitting the function of target sites that have only limited complementarity to the miRNA 5' end. The authors speculate that seed sites might be the

first functional sites acquired by protein-coding genes that require repression, and that additional sites might be acquired to promote stronger repression.

Based on their experimental results, Cohen and colleagues searched the *Drosophila* genome for biologically relevant targets, and estimate that the fly has about 100 sites for every miRNA in its genome. Since the fruitfly has anywhere from 96 to 124 miRNAs, that means it has 8,000 to 12,000 target sites (in the 11,000 genes sampled). This indicates

that miRNAs regulate a large fraction of protein-coding genes. Of the known animal miRNAs, many regulate critical developmental processes. This new approach to predicting targets should help reveal just how much regulatory control actually flows from these tiny bits of RNA.

Brennecke J, Stark A, Russell RB, Cohen SM (2005) Principles of microRNA–target recognition. DOI: 10.1371/journal.pbio.0030085

Recombination as a Way of Life: Viruses Do It Every Day

DOI: 10.1371/journal.pbio.0030117

In theory, a cell's nuclear membrane guards its contents by barring access to potential foes. In reality, pathogens employ a diverse bag of tricks to circumvent this barrier. The murine leukemia virus (a retrovirus), for example, waits until the nuclear membrane degrades during cell division. Other retroviruses, like HIV and so-called pararetroviruses, enlist protein escorts that help them slip through undetected.

Pararetroviruses include both animal viruses, such as hepatitis B, and plant viruses, such as the cauliflower mosaic virus (CaMV). Once inside the nucleus, the double-stranded DNA genome



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Turnip infected by cauliflower mosaic virus

of the CaMV is transcribed into an RNA transcript (called 35S RNA), thanks to the activity of the 35S promoter. (This CaMV promoter is widely used to drive transgenic expression in plants.) Replication proceeds through reverse transcription as a viral enzyme reverse transcribes the 35S RNA into genomic DNA that is then packaged into viral particles.

During replication, genetic material can pass between different viral genomes when two viral particles infect the same host cell. These exchanges can create novel viruses, much like mutations in bacteria can produce new bacterial strains that show resistance to host defenses and antibiotics. But with little data on viral recombination rates in multicellular organisms, it's unclear how these recombinant viral genomes are influencing

host infection. In a new study, Yannis Michalakis and colleagues follow the course of the cauliflower mosaic viral infection in one of its natural hosts, the turnip plant (*Brassica rapa*), to measure the frequency of viral recombination. Recombination was evident in over half of the recovered viral genomes, suggesting that recombination is routine for this plant virus.

It's thought that CaMV recombination occurs mostly outside the nucleus, in the host's cytoplasm, during reverse transcription. To quantify the frequency of such events, Michalakis and colleagues generated a CaMV genome with four genetic markers and then infected 24 turnip plants with equal amounts of marked and unaltered viruses. Recombination between the two "parent" genomes would produce viral populations with genetic material from both parents. The plants were harvested when full-blown symptoms developed, 21 days after inoculation, and viral DNA was extracted from their leaves to evaluate the occurrence and frequency of recombination.

Assuming that all marker-containing genomes could recombine, the authors predicted that the viruses should produce seven classes of recombinant genotypes, which is what they found. These recombinant genotypes showed up in over 50% of the viral populations—which the authors call an "astonishingly high" proportion. Though little information exists on the length of viral replication cycles in plants, the authors assumed a generation time of two days, which would amount to ten replication cycles over the 21-day experimental period. From this assumption, the authors calculated the recombination rate on the order of 4×10^{-5} per nucleotide base per replication cycle—hardly a rare occurrence. Certain CaMV genomic regions have been predicted as recombination hot spots, but the authors found that the virus "can exchange any portion of its genome... with an astonishingly high frequency during the course of a single host infection."

By evaluating the recombination behavior of a virus in a living multicellular organism, Michalakis and colleagues created a realistic approximation of recombination events during infection in the field. And since recombination events are linked to both expanded viral infection and increased virulence, understanding the rate of recombination could help shed light on mechanisms underlying the evolution and pathology of a virus—insight that could prove critical for developing methods to inhibit or contain an infection.

Froissart R, Roze D, Uzest M, Galibert L, Blanc S, et al. (2005) Recombination every day: Abundant recombination in a virus during a single multi-cellular host infection. DOI: 10.1371/journal.pbio.0030089

Separating Sisters: Shugoshin Protects SA2 at Centromeres but Not at Chromosome Arms

DOI: 10.1371/journal.pbio.0030098

DNA replication leaves the cell with two identical copies of each chromosome. To ensure their proper segregation during the anaphase stage of mitosis, the members of each pair, called sister chromatids, are held together by a protein complex, aptly named cohesin, that links the two not only at the centromere, but also along the chromatid arms. Anaphase is triggered when cohesin is cleaved, by the equally well-named separase. But cleavage is not the only way to remove cohesin from the chromosome; indeed, in humans and other higher eukaryotes, mitotic kinases such as Plk1 remove the majority of cohesin from chromosome arms—but not from the centromere—during prophase and prometaphase.

These facts raise two questions: what is the precise target of Plk1, and what protects centromeric cohesin from removal by the same pathway? Both questions are addressed in a new article in *PLoS Biology*. Jan-Michael Peters and colleagues show that phosphorylation of the cohesin subunit SA2, presumably by Plk1, is required for cohesin removal from chromosome arms in early mitosis, while data from Kim Nasmyth and colleagues suggest that a protein called shugoshin protects centromeric SA2 from such phosphorylation.

Cohesin is composed of multiple subunits, each of which can be phosphorylated at multiple threonine or serine amino acid residues. These subunits include Scc1 (the target of separase), Smc1, and Smc3, plus Scc3 in yeast, and SA1 or SA2 in humans and other higher eukaryotes. By isolating and analyzing cohesin subunits from cells undergoing mitosis, Peters and colleagues deduced that Scc1, SA1, and SA2 are phosphorylated only during mitosis, suggesting that phosphorylation of one or more of them triggers the breakup of cohesin. Further analysis by mass spectrometry allowed them to identify the exact amino acids that bore the phosphates on each subunit. In Scc1, these were clustered around the known sites of separase cleavage. The researchers showed that phosphorylation at these sites is required for efficient cleavage by the enzyme during anaphase, but is not required to dislodge cohesin specifically from the chromosome arms, as this proceeded essentially normally even after these sites were mutated to prevent their phosphorylation.

The same mutation strategy applied to SA2, on the other hand, revealed that phosphorylation of this subunit is essential for dissociating cohesin from the chromosome arms during prometaphase. Interestingly, the mutations did not prevent the

ultimate separation of the chromatids at anaphase. This suggests that separase, once it is activated, can cleave cohesin on the arms as well as at the centromere.

Cohesin at the centromere is removed later in mitosis than cohesin bound to chromatid arms, namely, at the metaphase-to-anaphase transition, suggesting centromeric cohesin is protected by a centromere-specific molecule. Possible candidates would be members of the shugoshin family, which are known to prevent unloading of centromeric cohesin during the first division of meiosis, thus keeping chromatids together as homologous chromosomes are separated.

To investigate human shugoshin's mitotic role, Nasmyth and colleagues depleted shugoshin by RNAi. The result was loss of cohesin not only from the arms but also from the centromere, early separation of chromatids, and failure of anaphase,

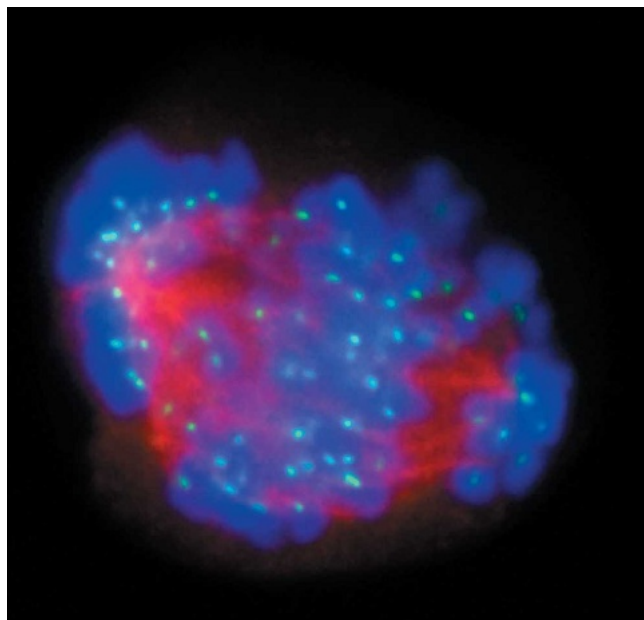
suggesting that shugoshin protects centromeric cohesin. But how? To find out, the authors examined the effect of shugoshin depletion in cells whose SA2 had been mutated to prevent phosphorylation. Strikingly, these cells underwent mitosis successfully. Together, these results suggest that shugoshin's normal mitotic role is to protect centromeric SA2 from phosphorylation, delaying chromatid separation until the moment when the chromosomes are ready to separate, at which time cohesin is cleaved by separase.

The picture that emerges from these two studies is that sister chromatid cohesion is safeguarded throughout early mitosis by shugoshin, which protects centromeric cohesin from the threat of protein kinases that, in the authors' vivid language, "maraud

mitotic chromosomes and threaten to destroy their integrity." This delicate balance of power between kinases and shugoshin means that any upset in the balance may prevent a cell from dividing properly, which often means not dividing at all. (Also see the Primer "Chromosome Cohesion: A Cycle of Holding Together and Falling Apart" [DOI: 10.1371/journal.pbio.0030094].)

McGuinness BE, Hirota T, Kudo NR, Peters JM, Nasmyth K (2005) Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. DOI: 10.1371/journal.pbio.0030086

Hauf S, Roitinger E, Koch B, Dittrich C, Mechtler K, et al. (2005) Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. DOI: 10.1371/journal.pbio.0030069



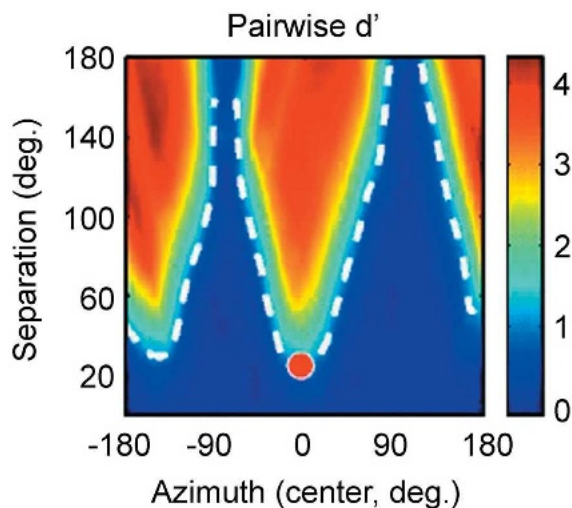
DOI: 10.1371/journal.pbio.0030098.g001

Premature loss of sister chromatid cohesion

How the Brain Signals a Sound Source

DOI: 10.1371/journal.pbio.0030103

Navigating one's environment requires sensory filters to distinguish friend from foe, zero in on prey, and sense impending danger. For a barn owl, this boils down mostly to homing in on a field mouse scurrying in the night. For a human—no longer faced with the reputedly fearsome saber-toothed *Megantereon*—it might mean deciding whether to fear rapidly



DOI: 10.1371/journal.pbio.0030103.g001

Discriminating sound locations from neural data

approaching footsteps from behind on a dark, desolate street.

How does the brain encode auditory space? The long-standing model, based on the work of Lloyd Jeffress, proposes that the brain creates a topographic map of sounds in space and that individual neurons are tuned to particular interaural time differences (difference in the time it takes for a sound to reach both ears). Another key aspect of this model is that

the location of a sound source is encoded by the identity of responding neurons.

Evidence for local coding of auditory space has been shown in the brains of owls and in a subcortical region of small mammals, but no such map has been found in the higher centers of the mammalian auditory cortex. What's more, electrophysiological recordings in mammals indicate that most neurons show the highest response to sounds emanating from the far left or right and that few neurons show that kind of response to sounds approaching head-on—even though subjects are best at localizing sounds originating in front of them.

Faced with such contrary evidence, other investigators have suggested that sound localization may rely on a different kind of code—one based on the activity distributed over large populations of neurons. In a new study, Christopher Stecker, Ian Harrington, and John Middlebrooks find evidence to support such a population code. In their alternative model, groups of neurons that are broadly responsive to sounds from the left or right can still provide accurate information about sounds coming from a central location. Although such broadly tuned neurons, by definition, cannot individually encode locations with high precision, it is clear

from the authors' model that the most accurate aural discrimination occurs where neuron activity changes abruptly, that is, at the midpoint between both ears—a transition zone between neurons tuned to sounds coming from the left and those tuned to sounds coming from the right. These patterns of neuronal activity were found in the three areas of the cat auditory cortex that the authors studied.

These findings suggest that the auditory cortex has two spatial channels (the neuron subpopulations) tuned to different sound emanations and that their differential responses effect localization. Neurons within each subpopulation are found on each side of the brain. That sound localization emerges from this opponent-channel mechanism, Stecker et al. argue, allows the brain to identify where a sound is coming from even if the sound's level increases, because it is not the absolute response of a neuron (which also changes with loudness) that matters, but the difference of activity across neurons.

How this opponent-channel code allows an animal to orient itself to sound sources is unclear. However auditory cues translate to physical response, the authors argue that the fundamental encoding of auditory space in the cortex does not follow the topographic map model. How neurons contribute to solving other sound-related tasks also remains to be seen.

Stecker GC, Harrington IA, Middlebrooks JC (2005) Location coding by opponent neural populations in the auditory cortex. DOI: 10.1371/journal.pbio.0030078

Engineering Gene Networks to Probe Embryonic Pattern Formation in Flies

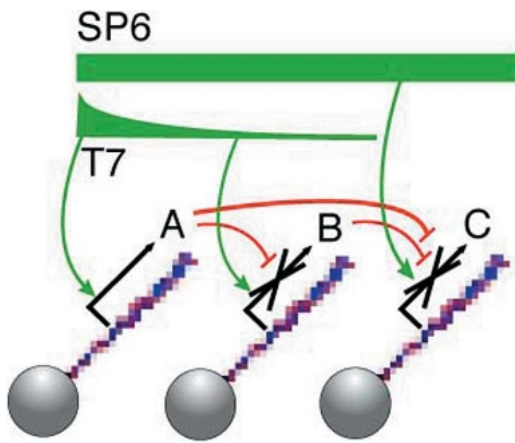
DOI: 10.1371/journal.pbio.0030104

When it comes to making the perfect body, it's all about expressing the right genes in the right place at the right time. This process begins even before the sperm and egg combine to form a zygote, because maternal factors are laid down in the egg that help establish the key axes of the body. After fertilization, precisely coordinated interactions between proteins called morphogens and a network of gene regulators establish a fly's anterior–posterior axis and its pattern of segments in just three hours.

In a new study, Mark Isalan, Caroline Lemerle, and Luis Serrano simulated segmentation patterning by creating a synthetic embryo and engineering an artificial version of the gap gene network, the first patterning genes expressed in the zygote. This simple system, combined with computer simulations to test network parameters, identifies significant features of the complex embryo and could do the same for other complex biological systems.

One of the first molecules to act is the

Bicoid protein. This morphogen is present in a concentration gradient—highest at the future head end. Different gap genes (so-called because their mutations create gaps in the segmentation pattern) respond to different levels of Bicoid, and are therefore switched on in different parts of the embryo. Expressed gap genes in turn modulate each other's activity. In the fruitfly, all of this action takes place while the embryo is a syncytium—having many nuclei but no cell membranes to separate them.



DOI: 10.1371/journal.pbio.0030104.g001

An artificial network to study patterning in development

Isalan et al. created a model of segmentation patterning by using a tiny plastic chamber containing various purified genes, proteins, metabolites, and cell extracts to mimic the gap gene network. Some of the genes were attached to magnetic microbeads, so that their location could be controlled by magnets anchored to the bottom of the chamber.

The authors investigated a number of open questions about pattern formation, including how a morphogen diffusing from a local source generates an expression pattern along a gradient and how transcriptional repression sets pattern boundaries. After testing the system to mimic a simple network of sequential gene transcription and repression, the authors increased the components and connectivity of the network, starting with systems that had no repression interactions and moving on to systems that had different levels of cross-repression. Patterns generated by networks involving repression were much different from those generated by networks lacking repression, fitting with observations that patterning boundaries in living flies require cross-repression.

But even the unrepressed system generated reproducible patterns, possibly caused by simple competition between the proteins. While such a situation likely bears little resemblance to that inside a fly egg, the authors suggest that any such competition effects would have to be tested in flies. In any case, this simplified approach can test hypotheses of how simple networks might evolve inside a cell. And since many aspects of *Drosophila* embryonic patterning remain obscure, these synthetic chambers will provide a powerful resource for testing different hypotheses.

Isalan M, Lemerle C, Serrano L (2005) Engineering gene networks to emulate *Drosophila* embryonic pattern formation. DOI: 10.1371/journal.pbio.0030064

Two-Way Traffic in B Cell Development: Implications for Immune Tolerance

DOI: 10.1371/journal.pbio.0030112

Development generally proceeds in one direction. Undifferentiated, pluripotent cells, which can become several different cell types, first of all become committed to restricted cell lineages. Then, under the control of developmental signals, committed cells gradually take on specialized characteristics, eventually producing mature, functioning cell types. To date, there has been little evidence to suggest that this process is ever reversed during normal development.

Now, however, Timothy Behrens and his colleagues report that the development of B lymphocytes, the antibody-producing cells of the immune system, can be switched into reverse by blocking or removing basal immunoglobulin signaling activity from immature B cells. Their findings have important implications for our understanding of how the immune system is tailored to respond efficiently to foreign antigens while ignoring self antigens and thus avoiding harmful autoimmune reactions.

B lymphocyte development, which occurs in the bone marrow, starts with the commitment of lymphoid progenitors to the B lineage and the somatic rearrangement of the heavy chain (HC) immunoglobulin (Ig) alleles. By stitching together diversity (D_H), joining (J_H), and variable (V_H) region DNA segments, many pro-B cells, each with a single but unique HC allele, are produced. Those cells in which the stitched-together HC allele encodes a functional protein undergo clonal expansion and proceed to the pre-B stage, before repeating the whole rearrangement process for the light chain (LC) Ig alleles. A productive LC rearrangement results in surface expression of IgM, which acts as the B cell receptor (BCR) for antigen for the immature B cell.

During development, any B cells bearing strongly self-reactive Ig receptors are removed—this process is called tolerization—either by clonal deletion, by functional inactivation, or by receptor editing. In this last process, new LC rearrangements revise the antigen specificity of the receptor. Little is known about the mechanisms driving receptor editing, but these new data from Behrens and colleagues suggest that signals provided by surface BCRs might suppress receptor editing in immature B cells.

To test this hypothesis, the researchers used a genetic system to remove the BCR from the cell surface of immature B cells in an inducible manner *in vitro*, and then compared gene expression patterns in these cells, control immature B cells, and pre-B cells. They discovered that the BCR-deleted cells had a gene expression pattern similar to that of pre-B cells, indicating that the BCR-deleted cells had gone back to an earlier stage of B cell development as a consequence of losing their BCR.

The researchers saw a similar effect on B cell differentiation state when they blocked downstream signaling from the BCR by the use of the tyrosine kinase inhibitor herbimycin A or the phosphatidylinositol 3-kinase inhibitor wortmannin. Finally, the researchers showed that cells undergoing “back-differentiation” also restarted LC rearrangement or receptor editing.

These data, suggest Behrens and co-workers, indicate that immature B cells actively maintain their developmental state by constitutive basal Ig signaling through protein tyrosine kinases. Their findings, they say, throw new light onto how receptor editing might be regulated in immature B cells in order to ensure that tolerance to self antigens develops. The researchers propose that when immature B cells encounter self antigens, down-regulation of surface Ig (BCR) via endocytosis might induce a back-differentiation program and thus induce an efficient editing response to the self antigen. Further experiments are now needed to show that these processes do indeed happen not just *in vitro*, as revealed here, but also *in vivo*.

Tze LE, Schram BR, Lam KP, Hogquist KA, Hippen KL, et al. (2005) Basal immunoglobulin signaling actively maintains developmental stage in immature B cells. DOI: 10.1371/journal.pbio.0030082

Forecasting the Path of a Raccoon Rabies Epidemic

DOI: 10.1371/journal.pbio.0030115

Rabies recently hit the national headlines when a Wisconsin teenager survived after showing full-blown symptoms. Even more remarkable, the girl—who was bitten by a bat—recovered after receiving a novel therapy, since doctors felt her case was too advanced for the standard rabies inoculations to work. Rabies is nearly always fatal if not treated immediately, and continues to pose a serious public health threat. Though most rabies fatalities in the United States stem from bat bites, far more people are treated for raccoon rabies.

A new strain of raccoon rabies started spreading throughout the eastern United States in the mid-1970s, after raccoons caught in Florida were released along the West Virginia–Virginia border to replenish hunting stocks. Some of the imports carried a rabies variant that caused an outbreak in local populations and has been steadily expanding ever since. In 1990, raccoons topped the list of most often reported rabid mammal.

Controlling this re-emerging public health threat depends on predicting the spatial dynamics of the disease—where new outbreaks might occur and how the virus might spread. Toward this end, Leslie Real and colleagues work on probabilistic simulation models that calculate the effects of various factors, such as local transmission rates between townships, ecological barriers to transmission, and long-distance “translocation” rates between townships. (The deliberately released Florida raccoons were one such translocation, but raccoons have also been known to hitch rides on garbage trucks.) As reported elsewhere, these models previously accurately reflected rabies spread in both Connecticut and New York. In a new study reported in *PLoS Biology*, Real and colleagues apply their model to the likely spread of rabies in Ohio—a potential gateway for spread throughout the Midwest—and find that raccoon rabies could spread throughout the state in just three years.

One strategy for limiting rabies spread is to establish vaccine corridors by distributing vaccine baits—vaccine doses hidden in fishmeal—to wild raccoons. This *cordon sanitaire* strategy limited rabies in Ohio to sporadic cases from 1997 until 2004, when a rabid animal was detected—11 kilometers beyond the buffer zone—in northeastern Ohio. The authors had previously shown that local transmission was significantly reduced when townships were separated by geographical barriers—the Connecticut

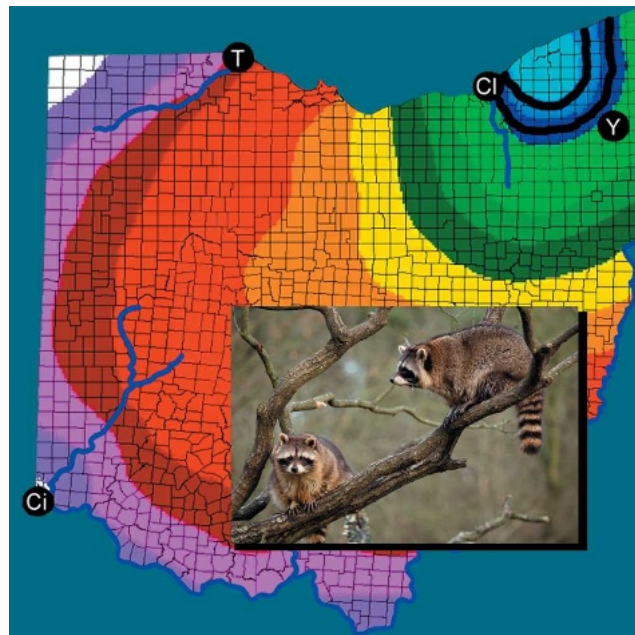
predict that rabies will spread far faster in Ohio than in New York and Connecticut.

Factoring in those garbage truck rides, the scenario is considerably bleaker: rabies would take just 33 months to spread across central Ohio—compared to 48 months to cross the much smaller state of Connecticut—and cover the state in 41 months. This transmission rate—100 kilometers/year—significantly surpasses previous estimates, which range from 30 to 60 kilometers/year. The potential for such rapid spread, if unchecked, “is quite alarming,” the authors warn. But they also point out that the path of a real epidemic would likely fall somewhere between these two scenarios, given the unpredictable nature of translocations. The authors also simulated potential breach points in the vaccine corridor and found that the Ohio and Muskingum rivers halted viral advance initially. But a raccoon can certainly cross a bridge when the opportunity arises, so any delays would likely be temporary.

Given the unpredictable nature of rabies transmission—challenging efforts to identify potential leaks in vaccine corridors and sites of dispersal—the authors’ simulations provide a valuable resource for anticipating alternate outbreak scenarios and preparing multiple game

plans to prevent or contain them. They also indicate the best sites for establishing a new vaccine barrier. And given how fast raccoon rabies could spread, Real and colleagues make a strong case that halting its western march depends on a strategy based on early detection and high-powered intervention programs—a sensible approach for any infectious disease.

Russell CA, Smith DL, Childs JE, Real LA (2005) Predictive spatial dynamics and strategic planning for raccoon rabies emergence in Ohio. DOI: 10.1371/journal.pbio.0030088



DOI: 10.1371/journal.pbio.0030115.g001

Predicting the spread of raccoon rabies across Ohio

River in Connecticut and the Adirondack Mountains in New York. In modeling the likely transmission path in Ohio, the authors incorporated the likely effect of Ohio’s five major rivers on transmission from local points along the Pennsylvania or West Virginia border.

Given Ohio’s topography (three of its rivers run along the southern and eastern border) and a single point of emergence in the northeast, the authors adjusted their simulations to estimate the potential impact of translocations. Even without the occasional garbage truck ride, because of the lack of ecological barriers in central Ohio, the simulations

Location, Location, Location: How Position Affects Gene Expression in the Nucleus

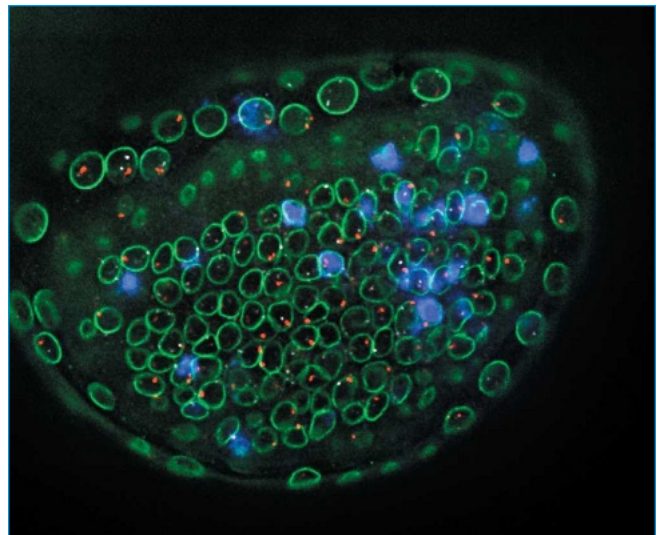
DOI: 10.1371/journal.pbio.0030106

Control of gene expression plays a role in determining cell fate, differentiation, and the maintenance of specific cell lineages. In the absence of regulation, aberrant gene expression can lead to developmental defects and disease. As a result, gene expression is highly regulated and that regulation takes many forms. Control mechanisms may be specific to one gene or operate on a gross chromosomal level, ultimately ensuring that genes are expressed at the right time, in the right place.

It is only in the run-up to and during cell division that chromosomes take on the condensed form that enables them to be recognized as discrete structures. During the rest of the cell cycle, interphase chromosomes exist in a relaxed state that at first glance looks like an unraveled ball of wool floating randomly about the nucleus. But a closer look reveals that they are in fact non-randomly organized and compartmentalized, and these groupings have functional ramifications for how genes are expressed or silenced (repressed).

What is actually visible is chromatin, a combination of naked DNA and proteins that associate with it. It can exist in two forms: euchromatin and heterochromatin. Actively expressed chromosomal regions (loci) are predominantly located within euchromatin, while loci within heterochromatic regions are silenced. Genetic and cytological evidence indicates that interaction between euchromatic genes and heterochromatin can cause gene silencing. Getting a gene into position for such an interaction may be achieved in two ways. The first is by changing the gene's position on the chromosome to bring it very close to expanses of centromeric heterochromatin, thereby increasing the likelihood for interaction. The second is by changing the position of a section of heterochromatin to place it close to a euchromatic gene. The small regions of heterochromatin involved in this second process seem sufficient to mediate long-range interactions between the affected gene and the larger heterochromatic regions near the centromere, but not so large or powerful as to mediate silencing by themselves. In this issue, Brian Harmon and John Sedat study the functional consequences of long-range chromosomal interactions—consequences that have been inferred in several different organisms but until now have not been analyzed on a cell-by-cell basis or directly verified.

Several *Drosophila* fruitfly mutants have been identified that exhibit cells in the same organ with varied phenotypes (appearance), though their genotypes (DNA instructions) are the same. This occurs through a phenomenon known as position-



DOI: 10.1371/journal.pbio.0030106.g001

Assessing gene expression and gene location in single cells

effect variegation, in which the expression of variegating genes is determined by their position on the chromosome relative to regions of heterochromatin. Working with fruitflies, the authors labeled three variegating genes and areas of heterochromatin with fluorescent probes and visualized expression of the affected genes in tissues where they are normally expressed. Silenced genes, they discovered, are far closer to heterochromatin than expressed genes, indicating that silenced genes interact with heterochromatin while expressed genes do not.

This study of interactions between a gene and heterochromatin in single cells illustrates unequivocally a direct association between long-range chromosomal interactions and gene silencing. The novel cell-by-cell analysis paves the way for further analysis of this phenomenon and will lead to a greater insight into the understanding and functional significance of nuclear architecture.

Harmon B, Sedat J (2005) Cell-by-cell dissection of gene expression and chromosomal interactions reveals consequences of nuclear reorganization. DOI: 10.1371/journal.pbio.0030067

Selection on Sex Cells Favors a Recombination Gender Gap

DOI: 10.1371/journal.pbio.0030099

Males and females of the same species can be strikingly different. Peacocks strut around with flashy feathers to attract mates, while peahens blend into their surroundings with more subdued colors. But differences are not always as obvious or easily explainable as in this classic example. Even the amount of genetic reshuffling that goes on during egg and sperm production differs between males and females in most species. An

evolutionary reason for this has eluded researchers since the phenomenon was originally discovered in fruitflies, Chinese silk worms, and amphipods almost 100 years ago.

Genetic diversity among organisms is promoted when genetic information is rearranged during meiosis, the cell division process that yields sperm and eggs (generically called gametes). During this genetic reshuffling, chromosome

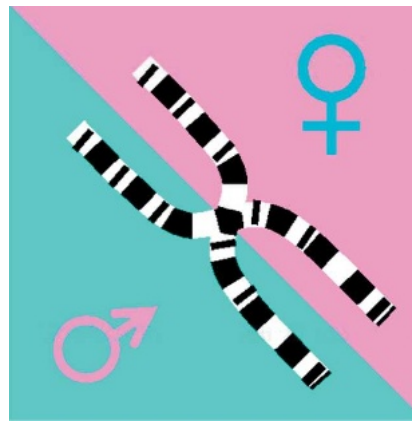
pairs overlap, forming structures called chiasmata ("crosses" in Greek), and physically recombine. This process does not just create diversity, it is also an example of diversity—recombination rates vary across chromosomes, sexes, and species.

An early 20th century hypothesis to explain the sex difference in recombination proposed that recombination is restrained within a pair

of unlike sex chromosomes (X and Y, for example) and that the suppression spills over to the rest of the chromosomes. Under this idea, the sex with dissimilar sex chromosomes (XY instead of XX, for example) should be the one with the least amount of recombination in all chromosomes. But that is not always the case. Some hermaphroditic species of flatworms, for example, lack sex chromosomes altogether but still display marked differences in male and female recombination rates. In one salamander genus, more reshuffling unexpectedly occurs in the sex with two different sex chromosomes.

In a new study analyzing an updated dataset of 107 plants and animals, Thomas Lenormand and Julien Dutheil bolster the argument against the recombination suppression hypothesis by showing that in species with sex chromosomes, the sex with two dissimilar sex chromosomes doesn't necessarily have a reduced recombination rate. Additionally, they found that, as a trait, the sex difference in recombination rate is not a lot more similar between two species in the same genus than between two species in different genera, suggesting that the difference evolves quickly.

An alternative hypothesis suggests that sexual selection might play a role in recombination differences. Reproductive success among males is often highly



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Male and female recombination rates differ

influenced by selection, so mixing up successful genetic combinations in males could be evolutionarily counterproductive. But in past studies, sexual selection was not related to variation in recombination rates.

Putting a new twist on this hypothesis, Lenormand and Dutheil realized that selection was not necessarily limited to the adult stage and that differences in selection among eggs or sperm might help account for recombination differences between the sexes. The authors reasoned that more opportunity for selection on sperm than egg should correspond to less recombination during sperm than egg production (and vice versa), consistent with the idea that

genetic combinations surviving selection should remain more intact in the sex experiencing the strongest selection at the gametic stage.

Though male gametes might be expected to be under stronger selection in many species, in true pines it seems to be the female gametes. The ovules compete with each other for resources over an entire year before being fertilized, and, indeed, from the dataset analysis, ovule production involves low recombination rates compared with male pollen in this group. In males, the opportunity for pollen competition was indirectly estimated using self-fertilization rates. The authors assumed that pollen grains competing for ovules of a self-fertilizing plant would be genetically similar and therefore experience less selection. Again, in the analysis, low selection correlated with less recombination in female gamete production, as predicted.

Is selection among eggs and sperm the evolutionary force generating sex-based variation in genetic shuffling? By demonstrating that differences may be influenced by gamete selection in plants, this work has added clarity to otherwise contradictory observations.

Lenormand T, Dutheil J (2005) Recombination difference between sexes: A role for haploid selection. DOI: 10.1371/journal.pbio.0030063

An Evolutionary Road Less Traveled: From Farming to Hunting and Gathering

DOI: 10.1371/journal.pbio.0030116

Invested with the arguably unique capacity for self-reflection, humans may well have asked the question, "Where did we come from?" ever since the dawn of self-awareness. From this universal question come origin stories as diverse as the cultures who tell them. In some cases, little is known about a population's evolutionary history aside from these stories—such is the case for the Mlabri people of Southeast Asia.

Until expanding agricultural development and modernization encroached on their forest homelands, the Mlabri lived mostly as nomadic hunter-gatherers in the forests of northeastern Thailand and western Laos. This lifestyle is unique among the other so-called hill tribes of Thailand—who all farm—raising the possibility that the Mlabri descended from the ancient Hoabinhian hunting-gathering culture of Southeast Asia and practice a way of life that predates agriculture.

Scant historical information exists on Mlabri language, culture, and origin, but Mlabri traditions speak to a long history as hunter-gatherers. The oral traditions of a neighboring hill tribe, the Tin Prai, paint a slightly different picture: several hundred years ago, legend has it, Tin Prai villagers sent two banished children downriver on a raft; the children, who survived by foraging in the forest, became the first Mlabri. In a new study,

Mark Stoneking and colleagues use the tools of molecular anthropology to investigate the agricultural versus hunting-gathering origin of the Mlabri and reveal a scenario remarkably similar to the traditional origin stories.

The notion that genetic analyses can shed light on this question, the authors explain, comes from a body of research indicating that hunting-gathering groups have a lower level of genetic diversity and a higher frequency of unique mitochondrial (mtDNA) sequence types than neighboring agricultural groups. In this study, Stoneking and colleagues compared the genetic diversity of the Mlabri with that of six other agriculture-based hill tribes by analyzing specific regions of each population's mtDNA, Y chromosomes, and autosomes (non-sex chromosomes).

mtDNA and Y chromosomes can help uncover clues to evolutionary origins because both are in effect haploid systems (i.e., there is only one copy of the Y chromosome and a lot of identical copies of mtDNA present in each cell), and so do not undergo recombination. This in turn means that observed genetic variations likely result from random mutation—which is assumed to occur at a predictable rate—allowing scientists to estimate the age of the genetic variation found in a population.

The mtDNA analysis revealed something remarkable: all the Mlabri mtDNA sequences were identical. Not only did all of the other hill tribes show “significantly higher” variation, but this lack of variation hasn’t been found in any other human population. The Y-chromosome and autosome analyses revealed the same reduced diversity, indicating a “severe reduction in population size” for the Mlabri. This reduction likely happened 500 to 800 years ago, Stoneking and colleagues conclude, and at most 1,000 years ago. But how? Since genetic analyses can’t distinguish between a population bottleneck and a founding event, the authors used simulations to calculate the amount of population reduction required to completely eliminate mtDNA diversity, arriving at “not more than two unrelated females” and “perhaps even only one.”

But were the first Mlabri farmers or hunter-gatherers? Unlike other hunting-gathering groups, the Mlabri share closely related mtDNA, autosomal, and Y-chromosome sequences with both the agriculture-practicing hill tribes and other agricultural groups

in Southeast Asia. Linguistic studies suggest that the Mlabri language arose after speakers of a related language, probably Tin, split off and came into contact with another, as yet unknown language, an event that likely happened less than 1,000 years ago.

The genetic and linguistic evidence indicates that the Mlabri were “founded” between 500 to 1,000 years ago by a single maternal lineage and one to four paternal lineages from an agricultural culture. With too few hands to farm, this tiny group likely turned to hunting and gathering. Altogether, Stoneking and colleagues conclude, these findings caution against automatically assuming that contemporary hunter-gatherer groups “represent the pre-agricultural lifestyle of human populations, descended unchanged from the Stone

Age.” Interestingly, the authors’ scenario of Mlabri origins is not so different from the story told by the Tin.

Oota H, Pakendorf B, Weiss G, Haeseler Av, Pookajorn S, et al. (2005) Recent origin and cultural reversion of a hunter-gatherer group. DOI: 10.1371/journal.pbio.0030071



DOI: 10.1371/journal.pbio.0030116.g001

The Mlabri of Thailand: Their history holds a lesson for anthropologists (Photo: Takafumi Ishida)