Research Digest

Synopses of Research Articles

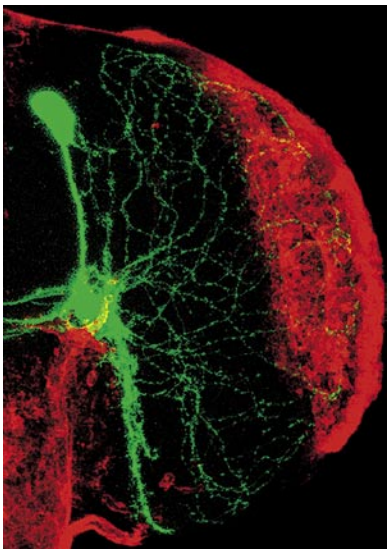
Biological Clock Depends on Many Parts Working Together

How do people subjected to the endless dark days of winter in the far northern latitudes maintain normal daily rhythms? Though many might feel like hibernating, a highly regulated internal system keeps such impractical yearnings in check. From fruit flies to humans, nearly every living organism depends on an internal clock to regulate basic biological cycles such as sleep patterns, metabolism, and body temperature. And that clock runs on similar molecular mechanisms.

Specific clusters of neurons in the brain are known to control the biological clock. Scientists believed these brain "clock cells" function as independent units. But new research described in this issue shows that the neurons do not act in isolation; rather, they collaborate with other neurons in a cell-communication network to sustain the repeating circadian rhythm cycles.

Clock cells within the brain maintain an organism's circadian rhythms, even in the absence of cyclical environmental signals like light, in a state scientists call "free running." Though it has long been clear that the circadian rhythms of an organism persist under such free-running conditions (for example, constant darkness), it was thought that the gene-expression patterns within the cells governing these biorhythms did not require any external, or extracellular, signals to continue ticking. In experiments described here, Michael Rosbash and his colleagues show that the key brain clock cells in fruit flies (*Drosophila*), called *ventral lateral neurons*, do indeed support the fly's circadian rhythms during periods of constant darkness and that the molecular expression patterns associated with these rhythms continue to cycle as well within other clock cells. These sustained expression patterns, however, require intercellular communication between different groups of brain clock cells.

In other words, the ventral lateral neurons do not act alone. When the molecular clock machinery was manipulated so that only the ventral lateral neurons were active, the fly's circadian rhythms were not sustained, suggesting the rhythms depend on other neuronal groups as well. The researchers also demonstrate that the persistence of normal cycling during constant darkness depends on a protein (called PDF) secreted by the ventral lateral cells.



***Drosophila* lateral neuron (green)**

The PDF neuropeptide protein was thought to connect the molecular expression pattern of the ventral lateral neurons with the manifestation of circadian rhythms, but the researchers found evidence of a larger influence. When mutant flies lacking a functional PDF gene were exposed to constant darkness, the molecular expression patterns gradually stopped. The scientists say this suggests that the ventral lateral neurons and the PDF protein it produces help coordinate the entire neural network that underlies circadian rhythms.

Peng Y, Stoleru D, Levine JD, Hall JC, Rosbash M (2003) DOI: 10.1371/journal.pbio.0000013

Large-Scale Association Study Confirms Genetic Complexity Underlying Type 2 Diabetes

A leading cause of death and disability, diabetes affects some 16 million Americans and up to 135 million people worldwide. While environmental factors such as diet and lifestyle are known to influence an individual's risk of getting adult-onset, or Type 2, diabetes, there is also a substantial inherited component, though many of the genetic pathways involved remain unidentified.

The challenge of defining these genetic pathways lies in the fact that diabetes is what is known as a "complex trait": not only is it likely that variations in many different genes or some combination of genes contribute to an increased risk, but there are probably different genes associated with diabetes in different populations. Tackling the monumental task of deciphering this genetic puzzle in the largest known study of its kind, Inês Barroso and colleagues confirm the genetic complexity of the disease and clearly demonstrate that untangling the genetics will require even larger studies.

In diabetes, defects in both the secretion and function of insulin—which is produced by the pancreas—impair the body's ability to metabolize glucose. Based on what is known about the biology of pancreatic function and diabetes, the researchers chose 71 potential suspect genes that could reasonably be expected to contribute to the disease if they malfunctioned. Some of these genes are involved in the function of pancreatic beta-cells, which secrete insulin; a second group influences the function of insulin and glucose metabolism; and a third plays a broader role in energy metabolism. The results show that none of the genes on their own had a large effect, though a number of gene variations increased risk slightly. They also suggest the existence of variations in several genes that influence the risk of Type 2 diabetes.

The dataset will be valuable for future studies of diabetes and supports the view that variation in genes affecting insulin production as well as insulin action can influence the risk of Type 2 diabetes. However, the genetic complexity of the disease—with a number of genes conveying a slightly increased risk—demands additional studies of larger populations to reliably identify the genes involved and the genetic variations that, alone or in combination, increase or lower an individual's risk of developing the disease.

Barroso I, Luan J, Middelberg RPS, Harding A-H, Franks PW, et al. (2003) DOI: 10.1371/journal.pbio.0000020

Functional Analysis of RSS Spacers

Based on sheer numbers, microbes should rule the world. Most don't cause disease, but those that do have the advantage of multiplying and mutating at a much faster rate than any multicellular organism can. So how does a slowly reproducing, trillion-celled organism like a human protect itself? By having the right weapon for the job—and that requires an incredibly diverse arsenal. A new study by a team of researchers from Yale University School of Medicine, Duke University Medical Center, and Mount Sinai School of Medicine demonstrates how the creation of that arsenal depends on a complex series of interactions between key genetic elements and proteins during the formation of the white blood cells called lymphocytes.

Two heavy hitters of the immune system—B and T cells—each produce unique protein receptors that specifically recognize and mediate the killing of the variety of potential foreign invaders, or antigens, such as bacteria, viruses, and parasites. (B cells make immunoglobulin, or antibodies, and T cells make T-cell receptors.) But these lymphocytes are unlike other cells: instead of making proteins from genes they inherited, they custom-make their genes by recombining fragments of their genes into new configurations. This genetic reshuffling process, called V(D)J recombination, yields the diversity of molecules necessary to combat the billions of different antigens they might encounter. The V, D, and J refer to different clusters of DNA sequences that follow specific rules of recombination.

While the products of recombination vary, the method does not. The fragments are spliced and then reassembled in a highly regulated process directed and controlled by a stretch of DNA (called a recombination signal sequence, or RSS) next to the gene fragment. The recombination process, the researchers show, relies on complex interactions among different parts of the signal sequences and the proteins that regulate them at key steps along the recombination pathway.

Each RSS is made up of three components: the nonamer, which controls the ability of proteins to bind to the gene fragments and initiate recombination; the heptamer, which directs the splicing of the gene fragment; and the spacer, which regulates how the gene fragments are recombined. Mutations in the DNA sequence of each of the three RSS components show that all play a critical role in the ability of the gene fragments to recombine appropriately.

While it has been established that spacers, as their name suggests, ensure that the space between the nonamer and heptamer is correct, the researchers show that spacers also regulate recombination activity by providing protein-binding sites along the DNA sequences that affect recombination. While the nonamer is the most important determinant of recombination, changes in the spacer, these researchers demonstrate, produced dramatic changes in the ability of the gene fragments to recombine.

Past studies have shown that recombination depends on the presence and sequence of specific nucleotides, but the quality of that recombination, the researchers say, can't be understood simply by analyzing those nucleotides in isolation. Generally speaking, highly conserved sequences have functional importance. But it would be a mistake, they suggest, to think that just because a nucleotide sequence isn't highly conserved, it's not biologically important. Using a computer model to predict how different protein-gene interactions affect recombination, the researchers demonstrate that a fuller understanding of the process depends on observing how all these elements—including those that aren't highly conserved—interact throughout the recombination process.

Lee AI, Fugmann SD, Cowell LG, Ptaszek LM, Kelsoe G, et al. (2003) DOI: 10.1371/journal.pbio.0000001

Developmental Origins and Evolution of *Buchnera* Host Cells

When it comes to exploiting a niche, endosymbionts take the prize. In endosymbiosis, one organism—the *endosymbiont*—invades the cells of another, in some cases taking up residence in a way that actually benefits the host. Bacteria are particularly adept at making themselves indispensable by insinuating themselves into some fundamental aspect of an organism's biology. The endosymbiotic hypothesis proposes that this is how certain eukaryotic organelles evolved from endosymbiotic bacteria. Insights into the mechanisms governing endosymbiosis will help biologists understand how this mutually beneficial relationship evolved and provide clues to one of the fundamental questions in biology: How did the eukaryotic cell evolve?

Aphid host of *Buchnera* endosymbionts

Over 10% of insect species rely on endosymbionts for their development and survival. In this issue, David Stern and colleagues look at one of the most studied pairs, the pea aphid and *Buchnera aphidicola*, and discover clues to the molecular foundation of their shared fate. (*Buchnera*, which can no longer survive outside its host cell, is thought to produce essential amino acids that the aphid cannot get on its own.)

While it is known that *Buchnera* are transferred from clusters of bacteriocytes in the mother to the adjacent early-stage embryo, it has been unclear how the bacteriocytes develop. Previous studies of the bacteria's genome have failed to explain the genetic basis of *Buchnera*'s ability to invade aphid cells. Consequently, Stern and colleagues have focused on the bacteriocytes, the specialized insect cells that house *Buchnera*, shedding light on the development of these cells as well as on the evolutionary adaptations in the aphid that made the bacteriocytes hospitable to *Buchnera*.

The researchers show that bacteriocytes differentiate and proliferate independently of *Buchnera*'s presence in the cell, and they identify three aphid transcription factors (proteins that regulate gene expression) that are expressed in three distinct stages during early-bacteriocyte development in the aphid embryo. The first protein is

expressed just before *Buchnera* enters the embryo; a second, as the bacteria invades; and a third, after the transfer is nearly complete. A second wave of the same transcription factors occurs at a later stage in aphid embryo development and increases the population of bacteriocytes.

This two-step specification of bacteriocytes, which occurs in related *Buchnera*-carrying aphid species, appears to be an evolutionarily conserved feature of aphids. It even occurs in an aphid species that once had a *Buchnera* endosymbiont and now has a yeast-like symbiont that lives outside the bacteriocytes. But this process is not observed in males of another aphid species that do not carry *Buchnera*. While traces of the first transcription factor activated in bacteriocytes are evident, the characteristic gene-expression pattern is not, and the aphids have no mature bacteriocytes.

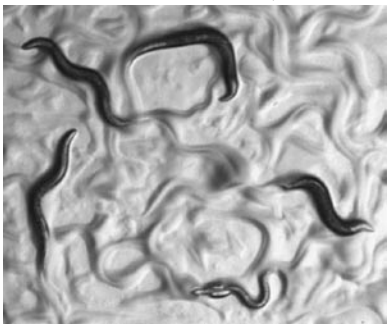
While it seems that the aphid has evolved new domains of expression in the bacteriocyte for these transcription factors—none of these transcription factors is expressed at a similar stage in other insects—the researchers cannot yet say whether these genes direct the specification of bacteriocytes. Still, these transcription factors are likely to play important roles in the bacteriocyte, suggesting that the union of aphids and *Buchnera* involved significant adaptations by the host.

Braendle C, Miura T, Bickel R, Shingleton AW, Kambhampati S, et al. (2003) DOI: 10.1371/journal.pbio.0000021

Supersensitive Worms Reveal New Gene Functions

The past ten years saw great progress in the field of molecular genetics, as new tools gave scientists the ability to investigate entire genomes instead of just one or two genes at a time. In this paper, Ronald Plasterk and colleagues developed a systemic approach using *Caenorhabditis elegans,* a tiny nematode and the first animal to have its genome sequenced, to gather functional information on nearly 400 genes.

Many of the systemic approaches to discovering gene function involve either measuring or deleting messenger RNA (mRNA), the molecule that helps translate genes into proteins. The method used here, called RNA interference, or RNAi, follows the deletion approach by taking advantage of a cellular process bearing the same name. In nature, RNAi is thought to be an important part of the innate defense machinery in plants and animals, protecting them from invaders like viruses by interrupting the manufacture of viral proteins. To do this, short double-stranded RNA molecules with complementary sequences to the target gene inhibit the gene's function by disabling mRNA, which effectively shuts down the gene. By mimicking this natural process to turn off selected genes, scientists can find clues to how those genes might normally function by



***Caenorhabditis elegans* worms**

watching what happens when they are taken out of the picture.

With the fully sequenced worm genome, it is possible to create interfering RNAs for all of its 20,000 or so genes. And because worms eat bacteria—which can themselves be used to deliver interfering RNAs—worms are the perfect RNAi model organism. The researchers fed the worms RNAi-producing bacteria, then observed the effects on the worm or its offspring to infer the function of the targeted gene. As previously reported, repeating this experiment for every gene in the worm genome, yields about 10% of the worms displaying abnormalities ranging from embryonic death to uncoordinated movement, suggesting defects in genes controlling development or muscle control, respectively.

Having previously identified an RNAi-hypersensitive mutant worm strain, Plasterk and his colleagues repeated the experiment in the mutants and report proposed functions for 393 previously unknown genes. The types of abnormalities observed in the short-lived mutations induced by RNAi, they say, resemble the more stable mutations seen in the collection of worm mutations cataloged by worm researchers over the years. Though the DNA alterations for many of these mutations are not yet known, researchers know roughly where they occur in the genome. And the researchers show here that they can use their RNAi experimental results along with what is known about the mutants to identify several of the sequence alterations. They also performed what is believed to be the first analysis in which independently generated large-scale RNAi results were systematically compared to see how variable such RNAi results are, and the results have implications for similar approaches not just in worms but in plants and other animals.

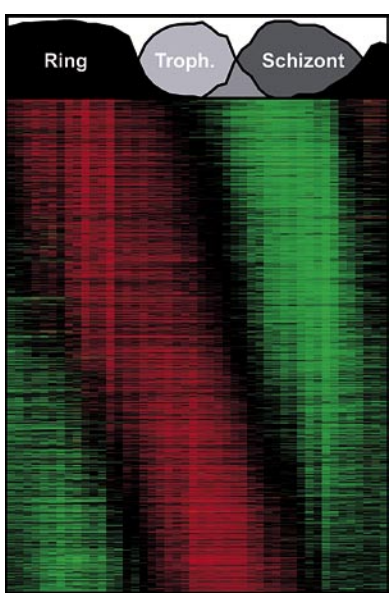
Simmer F, Moorman C, van der Linden AM, Kuijk E, van den Berghe PVE, et al. (2003) DOI: 10.1371/journal.pbio.0000012

Monitoring Malaria: Genomic Activity of the Parasite in Human Blood Cells

Every year, malaria kills as many as 2.5 million people. Of these deaths, 90% occur in sub-Saharan Africa, and most are children. While four species of the single-celled organism *Plasmodium* cause malaria, *Plasmodium falciparum* is the deadliest. Harbored in mosquito saliva, the parasite infects its human host as the mosquito feeds on the victim's blood.

Efforts to control the disease have taken on an increased sense of urgency, as more *P. falciparum* strains show resistance to antimalarial drugs. To develop new drugs and vaccines that disable the parasite, researchers need a better understanding of the regulatory mechanisms that drive the malarial life cycle. Joseph DeRisi and colleagues now report significant progress toward this goal by providing the first comprehensive molecular analysis of a key phase of the parasite's life cycle.

While *P. falciparum* is a single-celled



Gene expression profile of *P. falciparum*

eukaryotic (nucleated) organism, it leads a fairly complicated life, assuming one form in the mosquito, another when it invades the human liver, and still another in human red blood cells (erythrocytes). The intraerythrocytic developmental cycle (IDC) is the stage of the *P. falciparum* lifecycle associated with the clinical symptoms of malaria. Using data from the recently sequenced *P. falciparum* genome, the researchers have tracked the expression of all of the parasite's genes during the IDC.

The pattern of gene expression (which can be thought of as the internal operating system of the cell) during the IDC is strikingly simple. Its continuous and clock-like progression of gene activation is reminiscent of much simple life forms—such as a virus or phage—while unprecedented for a free living organism. Virus and phage behave like a "just-in-time" assembly line: components are made only as needed, and only in the amount that is needed. In this respect, malaria resembles a glorified virus.

Given the remarkable coupling of the timing of gene activation with gene function, as shown in this paper, this understanding could help identify the biological function of the 60% of genes in *P. falciparum* that encode proteins of unknown function.

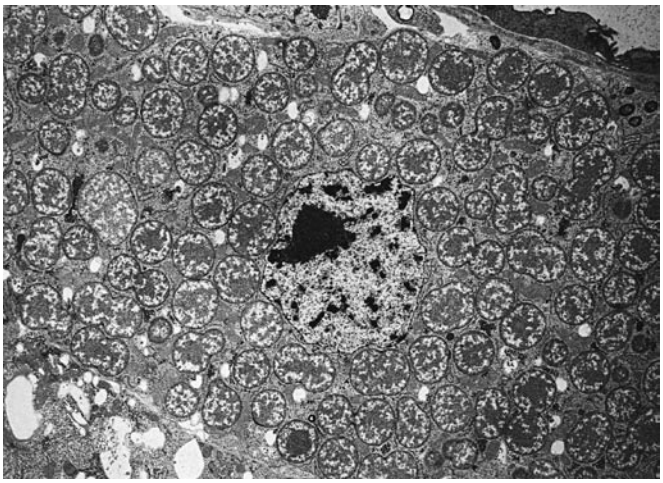
P. falciparum appears to be ultra-streamlined and exquisitely tuned to perform a single job: consume, replicate, and invade. The simple program regulating the life of *P. falciparum* may hold the key to its downfall as any perturbation of the regulatory program will likely have dire consequences for the parasite. This offers renewed hope for the design of inhibitory drugs targeted at the regulatory machinery that would irreparably foul the parasite's regulatory program, ultimately resulting in its death.

Bozdech Z, Llinás M, Pulliam BL, Wong ED, Zhu J, et al. (2003) DOI: 10.1371/journal.pbio.0000005

New Genomic Approach Predicts True Evolutionary History of Bacterial Genomes

Bacteria are an indiscriminate lot. While most organisms tend to pass their genes on to the next generation of their species, bacteria often exchange genetic material with totally unrelated species. That is why skeptics doubted that bacteria researchers could ever hope to map a reliable history of cell lineages in bacteria over time. But now, thanks to the availability of sequenced genomes for groups of related bacteria, researchers at the University of Arizona demonstrate that constructing a bacterial family tree is indeed possible.

Previous efforts to trace the ancestry of bacteria were constrained by a dearth of related bacterial genomes, which, among other things, prevented scientists from successfully accounting for bacteria's tendency to exchange genes with unrelated organisms. In this process, called *lateral gene transfer*, organisms acquire genetic material not from their ancestors, the most prevalent route, but from unrelated organisms. Lateral gene transfer greatly complicates the issue of who descended from whom, because two organisms could appear closely related based on the similarity of some genes but distantly related based on other genes. The problem is to determine which genes have been faithfully vertically transmitted—from parent cell to offspring—and thus reflect the history of the bacterial cell lineages.



Electron micrograph of Proteobacteria in eukaryotic cell

In this issue, Nancy Moran, Emmanuelle Lerat, and Vincent Daubin propose an approach that solves this problem by identifying a set of genes that serve as reliable indicators of the vertical transfer of bacterial cell lineages. This method has important implications for biologists studying the evolutionary history of organisms by establishing a foundation for charting the evolutionary events, such as lateral gene transfer, that shape the structure and substance of genomes. With this method, scientists can begin to understand how bacteria have evolved and how their genomes have changed.

Bacteria promise to reveal the most information about genomic evolution, because so many clusters of related bacterial genomes have been sequenced—allowing for broad comparative analysis among species—and their genomes are small and relatively compact. In this study, the researchers chose the ancient bacteria group Proteobacteria, an ecologically diverse group (including *Escherichia coli* and *Salmonella* species) with the most documented cases of lateral gene transfer and the highest number of species with sequenced genomes.

The researchers identified a set of likely single-copy orthologs (homologous genes that diverged due to the speciation of ancestral lineages) with widespread distribution in the different species of Proteobacteria that could be used to trace the history of the cell lineages. Surprisingly, they found that almost all of the 205 ortholog gene families they selected supported the same evolutionary branching pattern. Only two did not, which the researchers then investigated and found to be the result of lateral gene transfer.

These results, the researchers say, support the ability of their method to reconstruct the important evolutionary events affecting genomes. By mapping out the evolutionary path of genetic information on a genomic level, their approach promises to elucidate not only the evolution of bacterial genomes but also the diversification of species.

Lerat E, Daubin V, Moran NA (2003) DOI: 10.1371/journal.pbio.0000019

Borneo Elephants: A High Priority for Conservation

A new study settles a long-standing dispute about the genesis of an endangered species. With scant fossil evidence supporting a prehistoric presence, scientists could not say for sure where Borneo's elephants came from. Did they descend from ancient prototypes of the Pleistocene era or from modern relatives introduced just 300–500 years ago? That question, as Fernando et al. report in this issue, is no longer subject to debate.

Borneo elephant

Applying DNA analysis and dating techniques to investigate the elephants' evolutionary path, researchers from the United States, India, and Malaysia, led by Don Melnick of the Center for Environmental Research and Conservation at Columbia, demonstrate that Borneo's elephants are not recent arrivals. They are genetically distinct from other Asian elephants and may have parted ways with their closest Asian cousins when Borneo separated from the mainland, effectively isolating the Borneo elephants some 300,000 years ago.

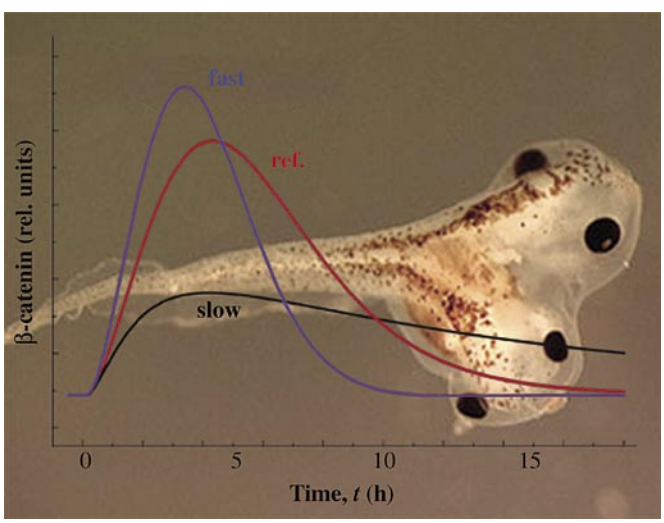
In the 1950s, Borneo elephants had been classified as a subspecies of Asian elephants (either Indian or Sumatran) based on anatomical differences, such as smaller skull size and tusk variations. This classification was later changed, partly because of the popular view that these animals had descended from imported domesticated elephants. Until now, there was no solid evidence to refute this belief and no reason to prioritize the conservation of Borneo elephants.

Their new status, as revealed by this study, has profound implications for the fate of Borneo's largest mammals. Wild Asian elephant populations are disappearing as expanding human development disrupts their migration routes, depletes their food sources, and destroys their habitat. Recognizing these elephants as native to Borneo makes their conservation a high priority and gives biologists important clues about how to manage them.

Fernando P, Vidya TNC, Payne J, Stuewe M, Davison G, et al. (2003). DOI: 10.1371/journal.pbio.0000006

Mathematical Modeling Predicts How Proteins Affect Cellular Communication

From the moment its life begins, the fate of a multicellular organism depends on how well its cells communicate. Proteins act as molecular switchboard operators to keep the lines of communication open and the flow of cellular messages on track. But charting the protein interactions, signaling pathways, and other elements that regulate these networks is no small feat. Previous efforts have been hampered by the lack of quantitative data—measurements of signal duration, amplitude, and fluctuation—on these regulatory pathways.



Understanding Wnt signaling through molecular modeling

Hoping to fill in some of the quantitative gaps, Marc Kirschner of Harvard Medical School, Reinhart Heinrich of Humboldt University Berlin, and colleagues developed a mathematical model as a framework for understanding the quantitative relationships among signaling proteins. To do this, they focused on a well-studied signaling pathway, the Wnt pathway, which plays a role both in various stages of embryonic development and in carcinogenesis. The researchers chose the Wnt pathway in part because a lot is known about it and in part because they could collect enough of the additional measurements they needed to build a solid model from experiments. And like most signaling pathways, Wnt is highly conserved. Consequently, developing tools that elucidate the Wnt pathway will not only provide insights into this important pathway, but have implications for understanding other communication pathways in animals from jellyfish to humans.

To get the additional measurements needed to build their model, the researchers reproduced aspects of the Wnt pathway in the cytoplasm of unfertilized frog eggs. Among the new data collected from these experiments were measurements of the concentrations of scaffold proteins, which bring other components in a pathway together by providing an interaction surface. Strikingly, they found that the principal scaffold proteins involved in the pathway, axin and adenomatous polyposis coli (APC), occur in dramatically different concentrations and perform their jobs in different ways. After a series of refinements based on additional experiments, the model could not only simulate the behavior of the main players in the pathway—both in the absence and presence of a Wnt signal—it also suggested why the two scaffold proteins are present in different concentrations. Axin occurs at very low concentrations relative to the other proteins in the pathway and is likely to bind with them randomly, while APC occurs in similar concentrations and probably binds with the other components in an ordered manner. Because the proteins axin interacts with are also involved in other signaling pathways, the authors propose that the low level of axin here may help the pathways retain their modularity, preventing the Wnt pathway from interfering with the other pathways.

These findings demonstrate that modeling can offer powerful new insights into the workings of complex signaling systems,

cutting through the static to pick up important signals even in those pathways that are well understood. The results have important implications for developmental biology and human disease: The Wnt pathway is often activated during carcinogenesis—and mutations in several of these signaling proteins have been linked to colon cancer—suggesting that cancer can develop when signals in the Wnt circuitry somehow get crossed. By predicting how quantitative factors may influence the behavior of signaling networks, mathematical models such as this could shed light on the role that breakdowns in cellular communication play in carcinogenesis. The researchers argue that future attempts to characterize these complex networks must incorporate quantification measurements, and their modeling efforts suggest ways to do that.

Lee E, Salic A, Krüger R, Heinrich R, Kirschner MW (2003) DOI: 10.1371/journal.pbio.0000010. ■