

NewsBytes

Decoding Promotion

Despite their identical genomes, cells in the body develop distinct personalities—become neurons or liver cells, for instance—due to differences in gene expression. The mechanism that regulates this process has remained obscure, but a new study explains it using a simple thermodynamic model.

“Much of this phenomenon can be explained by a simple model of protein-protein and protein-DNA interactions,” says principal author **Barak Cohen, PhD**, of the Washington University Medical School in St. Louis. “In our system there is no need to account for complicated chemical processes.”

According to large-scale studies of eukaryotic genomes, gene expression is turned up and down when transcription factors interact with a zone of noncoding DNA located upstream from the gene—the gene’s promoter. This interaction is complex, and can involve a variety of transcription factors operat-

ing in concert. Indeed, a typical promoter may include 20 or more sites that can each bind any one of about 250 known transcription factors. The number of possible promoters and their interactions is thus enormous, but data about their behavior is limited to a few thousand known promoters. “This makes it real hard to tease out the rules of gene regulation,” says Cohen.

To make the problem tractable, Cohen and his collaborators built 2800 synthetic promoters each combining three to five transcription binding sites from about 20 known sites. Experiments on yeast cells showed that varying these mini-promoters for a gene yielded nearly three orders of magnitude variation in its expression. To analyze the promoter-expression relationship, the researchers invoked a thermodynamic model developed in earlier studies. In this model the interactions between proteins and their binding sites either help or hinder the recruitment of RNA polymerase—the molecule needed to build RNA from the DNA—to the promoters. The researchers “trained” the model using measured gene expression levels for a set of about 400 promoters, and tested it on an independent set of another 83 promoters.

The trained thermodynamic model explained nearly 50 percent of the variation in gene expression for the training set, and about 44 percent of the variation for the independent set. In contrast, empirical models relying on genomic data explain less than 25 percent of the variation in gene expression, says Cohen. The system also showed how weak binding sites cooperate to regulate gene expression, an effect that prior models failed to address. When applied to actual yeast genome data, the system found that Mig1, a transcription factor associated with glucose metab-

olism, regulated several additional genes not previously known to be regulated by this protein. “This is remarkable because Mig1 is one of the most widely studied transcription factors,” says Cohen.

In addition to shedding light on gene regulation, the findings could also facilitate *in silico* engineering of promoters with completely novel expression patterns, says Cohen. Such custom-designed promoters could be a boon for stem cell development, tissue engineering, regeneration, and similar areas. As a step towards this goal, the researchers plan to extend their work to mammalian cells, Cohen says.

“This paper is an important advance developing quantitative models for transcriptional regulation,” says **Eran Segal, PhD**, of the Weizmann Institute of Science in Israel. “It shows on a large scale what has been demonstrated previously on smaller sets of genes in fly and bacteria.” **Paturu Kondaiah, PhD**, of the Indian Institute of Science in Bangalore agrees with this assessment, but points out that transcription factors behave differently depending on their conformation, and can also recruit co-activators or co-repressors. “The next step is to take these effects into account,” he says.

—By **Chandra Shekhar, PhD**

The Brain in Transition

Patients with schizophrenia and other psychotic disorders are known to have adverse brain changes, such as reduced volume—but it’s unclear what comes first, the disease or the abnormality. Now, for the first time, researchers have shown that the brain is actually shrinking as psychosis unfolds. The results appear in the January 10 issue of *Schizophrenia Research*.

“We found that people who go on to develop psychosis have a different profile of neuroanatomical changes than those who do not,” says **Tyrone D. Cannon, PhD**, professor of psychology, psychiatry, and biobehavioral sciences at the University of California, Los Angeles. The findings may have implications for predicting and preventing psychosis.

Cannon and his colleagues took pre-



Courtesy of **Barak Cohen**; graphic by **Michela Hunt**.

“Our approach allowed us to detect more subtle anatomical changes in the brain, which is critical because we would not expect the changes associated with onset of psychosis to be so gross as to be detectable using standard voxel-based methods,” Tyrone Cannon says.

morbid brain MRI images of 35 individuals who had never had a psychotic episode but were considered at “ultra-high” risk based on early symptoms or a strong family history. They re-scanned their brains after an average follow-up of 1.3 years—during which time 12 developed psychosis.

Previous studies had considered losses in brain tissue density, a voxel-level measure of brain volume (typical resolution on the order of 1 cubic millimeter).

Cannon’s team used a higher-resolution measure of volumetric brain change—the brain contraction rate. This parameter is calculated by transforming MRI scans into 65,000-point maps of the brain’s surface and determining how fast the surface points are contracting between sequential scans. They found that the prefrontal lobes of subjects who progressed to psychosis were contracting significantly faster—by about 0.2 millimeters per year—than those of subjects

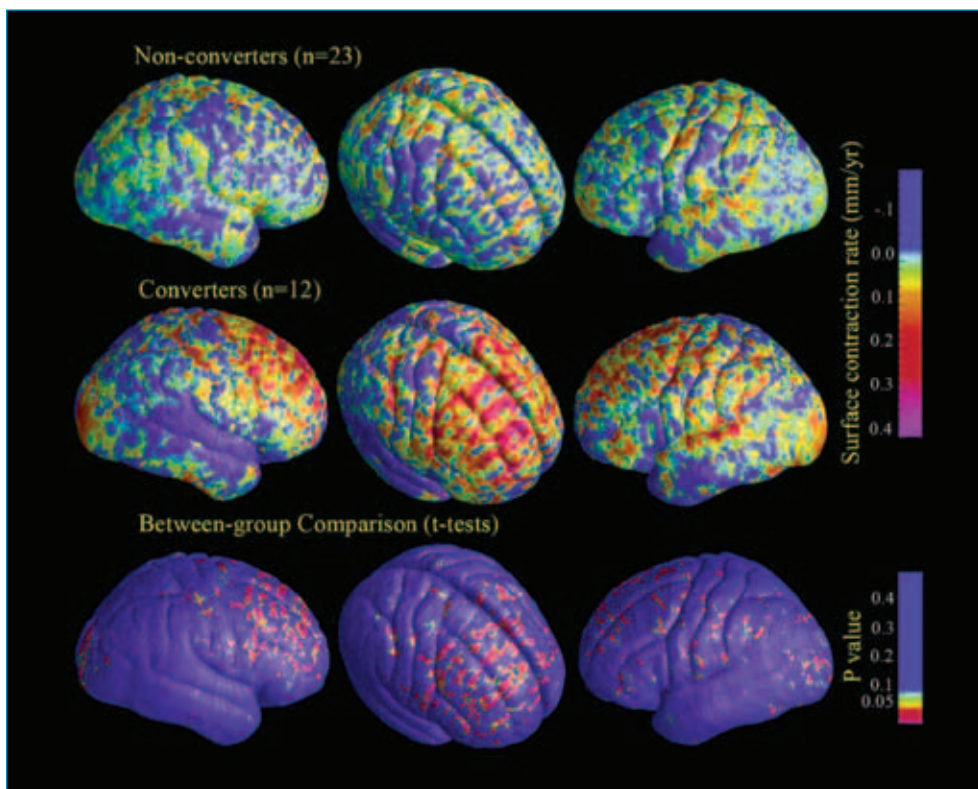
who did not progress. “Our approach allowed us to detect more subtle anatomical changes in the brain, which is critical because we would not expect the changes associated with onset of psychosis to be so gross as to be detectable using standard voxel-based methods,” Cannon says.

Though the study is interesting, it is small and lacks a healthy control group—which makes it difficult to tell how much of the detected changes are due to random variation and normal aging versus disease, comments **R. Grant Steen, PhD**, associate professor of psychiatry at the University of North Carolina School of Medicine. Also, it took an average of eight months to re-scan subjects with disease after their initial psychotic episode, so the timing of the changes is not entirely clear and could be related to treatment, he says.

Medication is an unlikely explanation since its use was limited and unrelated to brain contraction rates, Cannon replies. Still, he agrees, “the full significance of the findings awaits confirmation in large, multisite, longitudinal imaging studies that are currently underway.”

If the changes observed do turn out to be a cause of the onset of schizophrenia and associated disorders, “it may eventually be possible to provide treatment in high risk individuals—to delay or prevent the onset of psychosis,” Cannon concludes.

—By *Kristin Sainani, PhD*

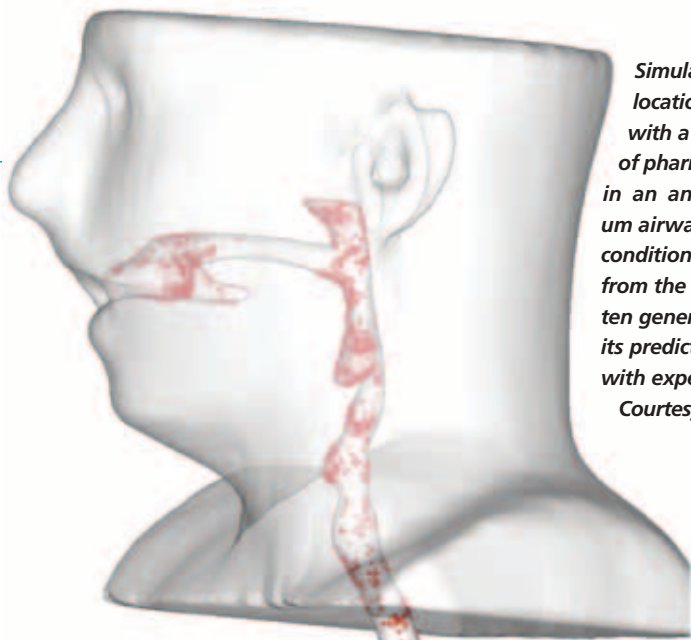


Shrinking Brains. The top panels show the average rates of surface contraction in different regions of the brains of 12 high-risk subjects who went on to develop psychosis (converters) and 23 who did not (non-converters). Red and pink regions are contracting the fastest. The bottom panel shows regions where the converters’ brains were contracting significantly faster than non-converters’. Yellow, red, and pink regions had the most statistically significant differences. Reprinted from Sun, D., et al., *Progressive brain structural changes mapped as psychosis develops in ‘at risk’ individuals*, *Schizophr. Res.* (2009) 108(1-3):85-92.

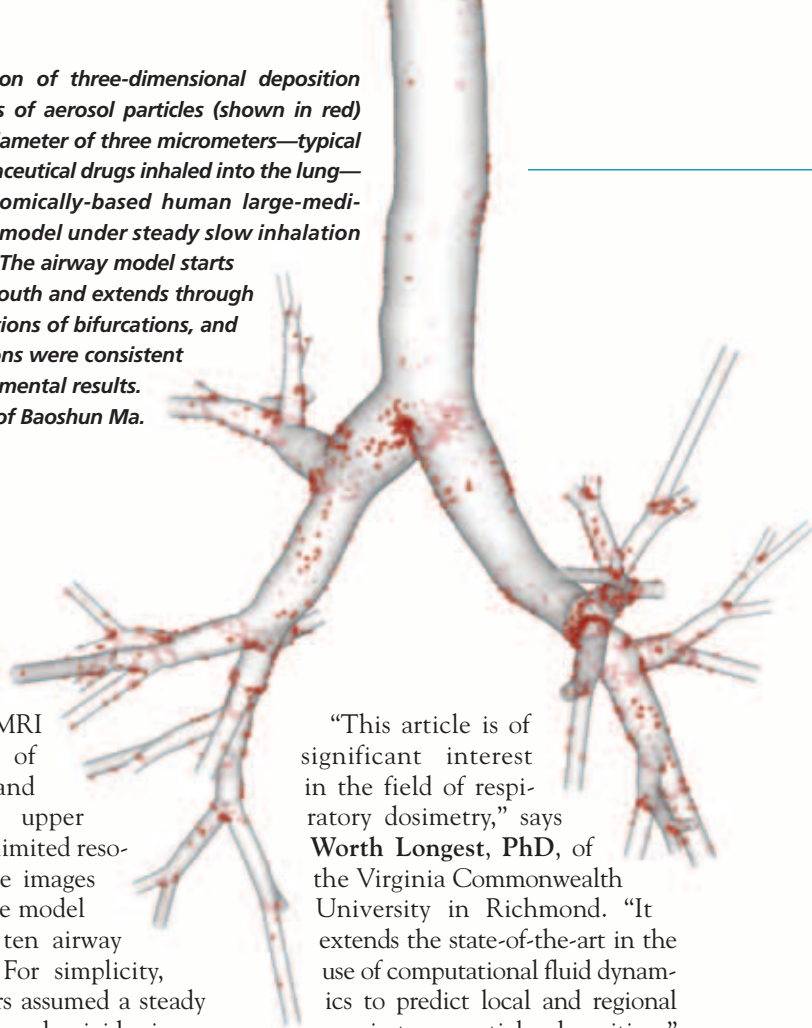
The Fate of Inhaled Particles

New computational model simulates how particles in the air get deposited in the lungs during breathing

Depending on their nature, microscopic particles suspended in air—



Simulation of three-dimensional deposition locations of aerosol particles (shown in red) with a diameter of three micrometers—typical of pharmaceutical drugs inhaled into the lung—in an anatomically-based human large-medium airway model under steady slow inhalation conditions. The airway model starts from the mouth and extends through ten generations of bifurcations, and its predictions were consistent with experimental results.
Courtesy of Baoshun Ma.



called aerosols—can cause or treat disease when inhaled. A key factor in both cases is how the particles accumulate throughout the respiratory system. A new study uses fluid dynamics and an anatomically accurate human airway model to simulate this process, potentially paving the way for improved disease understanding and patient-specific drug delivery.

“It is one of the first computational studies that uses anatomically correct models to predict aerosol deposition,” says principal author **Kenneth Lutchen, PhD**, of Boston University, principal author of the study published in the February 2009 issue of *Annals of Biomedical Engineering*.

Starting with the windpipe, airways in the human respiratory system branch out, producing about 23 levels of branching or “generations.” The resulting structure includes nearly 10 million microscopic airways, making it hard to study aerosol deposition. According to Lutchen, experimental methods relying on *in vivo* rat studies or lung-shaped casts have yielded useful, but preliminary, data. Prior computational studies have dealt with more complex respiratory structures, but typically used idealized lung models instead of the actual anatomy. Further, many of them ignore the upper airways where most of the deposition occurs, Lutchen says.

In contrast, Lutchen and his collaborator **Baoshun Ma, PhD**, modeled

their lung from MRI and CT images of healthy men and included the upper airway. The limited resolution of the images restricted the model to the first ten airway generations. For simplicity, the researchers assumed a steady flow of air through rigid airways instead of a natural breath pattern. They then used a computational fluid dynamics framework with a standard turbulence model to simulate aerosol deposition for different particle sizes and airflow rates. Results indicated that large particles (with a diameter of 30 micrometers—about the width of a human hair) end up mostly in the mouth and upper throat, whereas small (1 micrometer) particles typical of pharmaceutical drugs inhaled into the lung spread out more evenly. Typically, the left lung absorbed more particles—as much as 5 times more for some parameter settings—compared to the right lung. “These predictions are consistent with experimental data,” says Lutchen.

Inhaled aerosols have emerged as an important method for delivering drugs for lung-related conditions ranging from asthma to cystic fibrosis. However, proper dosing requires accurate, patient-specific prediction of aerosol deposition patterns under a variety of conditions. Lutchen hopes that the new approach will eventually facilitate this task. “This model will tell you what particle sizes and inhaled volumes you need to get the desired dose for a specific patient,” he says.

“This article is of significant interest in the field of respiratory dosimetry,” says **Worth Longest, PhD**, of the Virginia Commonwealth University in Richmond. “It extends the state-of-the-art in the use of computational fluid dynamics to predict local and regional respiratory particle deposition.”

To be of use in clinical applications, however, the system should be extended to include transient effects over a breathing cycle, effects of airway wall motion, and a more robust turbulence model, he adds.

—By **Chandra Shekhar, PhD**

RNA Families Set Up House in Wikipedia

For scientists submitting to the journal *RNA Biology*, the publishing guidelines now include a new task: Submit a Wikipedia entry. In collaboration with the RNA database Rfam, the journal recently launched a new section, RNA Families, that requires a corresponding peer-reviewed Wikipedia article along with each article published in the section.

“It is so globally important to have knowledge accessible to everybody,” says **Renée Schroeder, PhD**, editor-in-chief of the journal.

The new section, dedicated to descriptions of non-coding RNA families, debuted in the January/February/March issue of the journal, with one article and its corresponding Wikipedia entry. The entries are not meant to exactly mirror the scientific literature, Schroeder says. “In a research article

you have the way you acquired the knowledge, and in Wikipedia you have the results,” she says.

This is the first instance of such a link between Wikipedia and a scientific journal, says **Alex Bateman, PhD**, co-director of Rfam, an open-access database of non-coding RNA families coordinated by the Wellcome Trust Sanger Institute in Cambridge, UK. In 2007, Bateman and his colleagues linked the database to Wikipedia.

ly translate to the encyclopedic format, and the site is meant to be a source of accurate information, so “there shouldn’t be too much that hasn’t been tested and retested,” she says.

The Rfam and *RNA Biology* entries fall under the rubric of the Molecular and Cellular Biology wiki project, which is working to improve all molecular biology, biochemistry and cellular biology entries. **Tim Vickers, PhD**, director of the wiki project and postdoc-

Predicting Vaccine Efficacy

Researchers developing a new vaccine currently have no direct way of predicting its efficacy short of exposing patients to the disease. A new study that combines gene expression data with advanced computational analysis provides the first evidence that the vaccine-induced immune response can be predicted.

“We develop vaccines but can never say how effective they will be,” says **Bali Pulendran, PhD**, a researcher at the Emory Vaccine Center in Atlanta who led the study, published in *Nature Immunology* in November 2008. “Only after exposure do we really know.”

To gauge a vaccine’s effectiveness, scientists evaluate indicators of the so-called “adaptive” immune response, which develops over time. The titer—a measurement of the concentration—of long-term antibodies in the blood is one indicator. The number of killer T cells is another. But a more complete profile of the early or “innate” immune system reaction could help researchers screen vaccine candidates or help identify individuals whose adaptive immune systems don’t respond.

To develop such a profile of the immune response, Pulendran and his colleagues monitored patients given the yellow fever vaccine—a vaccine that has been given to more than 600 million people and is considered one of the most powerful ever developed, proving effective 80 to 90 percent of the time. In two sets of volunteers (15 in the first group and 10 in the second) Pulendran’s group sought to correlate patients’ innate (early) immune response to the vaccine with the later T cell response. Several cytokines and 65 genes responded to the vaccine in significant ways, but there was no apparent link between this innate signature and the subsequent T cell reaction.

To zero in on what was evidently a subtle connection, the researchers looked more broadly at the gene expression signatures for the first set of patients. They found 839 genes whose expression correlated with the T cell response. Using these data and a supervised learning algorithm developed by **Eva Lee, PhD**, at the

“Obviously we scientists all like to publish papers, but if you just do that and don’t reach out and tell people why your work is important, that’s a big chunk missing,” Tom Vickers says.

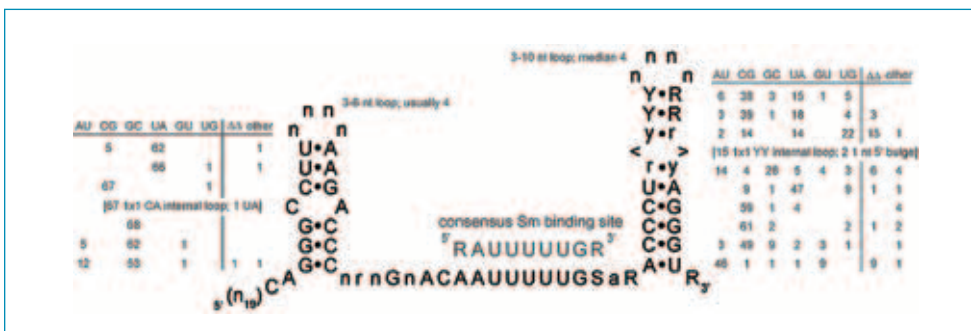
Editing of the Wikipedia articles automatically updates the database. In fall 2008, he brought his idea for a new publishing paradigm to *RNA Biology*.

Bateman thinks this is an exciting step for a scientific journal. “It wouldn’t be reasonable to claim that these articles were going to change the world,” he says. “But the important thing is that the model is really interesting. Hopefully this can be an experiment that other journals can follow in other areas of science.”

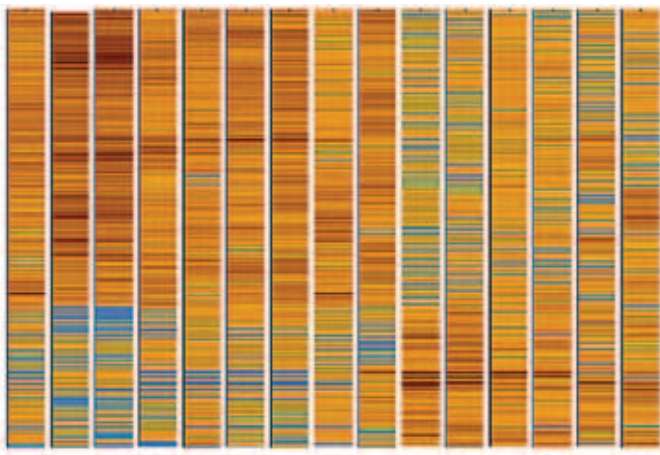
Schroeder points out that this model won’t work for every scientific journal. The article subjects must easi-

toral fellow at Washington University, thinks the decision by *RNA Biology* is a step in the right direction toward getting more scientists involved with updating and maintaining Wikipedia. “Obviously we scientists all like to publish papers, but if you just do that and don’t reach out and tell people why your work is important, that’s a big chunk missing,” he says. “Editing Wikipedia and giving the general public a good summary of the science in your field, that’s almost as important as publishing scientific papers.”

—By Rachel Tompa, PhD



A figure from the first Wikipedia entry tied to an *RNA Biology* article, entered into Wikipedia in November 2008. The entry and article, published in the January/February/March 2009 issue of *RNA Biology* describe the SmY family of non-coding RNA molecules found in some nematode species.



The researchers used correlation cluster analysis of expressed genes to confirm that subjects could be sorted clearly into two categories: "high" or "low" responders to the vaccine, based on the strength of T cell response. Courtesy of Bali Pulendran. Reprinted from Querec TD et al., *Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans*, *Nature Immunology* (2009) 10(1):116-125 with permission from Macmillan, publishers.

Georgia Institute of Technology, they pulled out eight different genetic signatures from data from the first group that strongly predicted the T cell response in the second group of volunteers. The researchers also used that algorithm to generate signatures predicting the titer of long-term antibodies.

In the case of both T cells and antibodies, the researchers were particularly interested in a small number of genes that featured in all the predictive signatures. These genes form a core set that doctors could potentially monitor to predict how effective a vaccine will be in a patient. Pulendran also hopes that by working to replicate the innate reaction to yellow fever, scientists may be able to make potent vaccines against other pathogens.

"If the approach could be extended to development of vaccines against different sorts of pathogens, it would be a real advance," says **Larry Stern, PhD**, an immunologist at the University of Massachusetts. "The key here is whether the same signature would be induced by other pathogens," he says, noting that even if the method works only for related pathogens, such as dengue fever and West Nile virus, that would still be a very valuable contribution.

—By **Kaspar Mossman, PhD**

A Model Neuron

For patients suffering from nerve damage, neural regeneration is a faint hope. It rarely happens naturally, and attempts to coax new growth often fail. Researchers are trying to develop scaffolds to guide regenerating neurons in the body. But the best way to guide neural growth on these

substrates remains unknown. So *in vitro* studies of neuronal behavior on these templates are a key first step. But such studies largely rely on trial and error rather than engineering principles.

Now, scientists have developed a computational model to predict the first stage of neural development, neuron polarization. Their model, published in the February issue of *Annals of Biomedical Engineering*, could yield powerful predictions for better scaffold design in neural tissue engineering.

"Our work is unique as it is the first effort of its kind to quantitatively model the interactions of the neuron with the substrate," says **Muhammad Zaman, PhD**, assistant professor of biomedical engineering at the University of Texas at Austin.

Directing neuron growth on an artificial substrate is no easy feat. To lead to nerve regeneration, the neurons must polarize in the same direction, but immature cells send out multiple tendrils in all directions initially. The projection that grows the longest eventually becomes the axon, the path for sending out electrical signals; the others become dendrites, the stimulus receptors for the neuron. These projections' fates can be influenced by various external cues, both chemical and physical.

For unknown reasons, physical factors such as ridges dominate over chemical cues *in vitro*. That is, if an immature neuron is faced with chemical cues on one side and ridges on the other, it will tend to polarize toward the ridged side, extending its axon along one of the grooves.

To model the cell's reaction to its surroundings, Zaman and his colleagues broke neuron polarization into several small steps, using probabilities at each step to predict the cell's next choice in projection growth. They introduced parameters based on known factors, such as the physics of the internal forces acting on the projections, how projections behave on different substrates and how they react to different chemical cues.

Their model accurately reproduced known results, and also revealed that ridge size is important to a neuron. If the ridges are too small or too wide, the neural projections view them as a continuous surface, and chemical cues will win out. For the kinds of cells in Zaman's experiments, the best ridges were between two and 10 microns wide.

"The cells seem to like persistence," Zaman says. Once a projection starts down a ridge, it is like a car on a one-way road. With only one direction to travel, growth is much faster. But if the ridge is too wide or too narrow, the cell no longer sees the road.

"There is a lack of engineering rigor in the whole area of tissue and regenerative engineering," says **Gabriel Silva, PhD**, assistant professor in bioengineering at the University of California, San Diego. "I think the approach that these authors have taken is exactly what's needed, which is a systematic, quantitative, rigorous engineering-type model that can guide the design of experiments and materials."

—By **Rachel Tompa, PhD** □

A single rat neuron has a decision to make. When this cell was immature, it was placed on an artificial substrate in between immobilized nerve growth factor (on the left) and a surface of two micron-wide ridges (on the right). The projection that eventually turned into an axon grew along the ridged side. Zaman's computational model of neuron growth reproduced this outcome and also identified axons' preferred ridge size. Photo courtesy of Natalia Gomez, PhD, formerly at the University of Texas at Austin.

