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Why We Swing

Most people swing their arms when they walk. Indeed, like several characters in a classic Seinfeld episode, we're surprised when they don't. Yet we don't really need to swing our arms in order to move forward, as we all know when we carry a box with both hands. So why do we swing our arms when we walk? A recent computational model by **Jaeheung Park, PhD**, a re-searcher at the Stanford Artificial Intelligence Laboratory at Stanford University, provides some insight.

Arm swinging, Park hypothesized, serves the same purpose as rotational friction—the friction between the foot and the ground that keeps our feet from turning in or out like windshield wipers. And his simulations, published in the *Journal of Biomechanics* in April 2008, confirmed that possibility.

In the past, many biomechanical models of gait have omitted the arms. But as such models strive for greater realism, it has become more important to account for secondary movement by the arms. One way to do that is to simulate the trajectories of the arms and joints. But Park took a different "task-oriented" approach adapted for human simulations from his thesis advisor's work on industrial robots.

In his simulations, Park instructed the feet to perform a task—"walk"—but gave no instructions to the arms. Then he varied the amount of rotational friction between the foot and the ground. When the rotational friction forces experienced by the model's foot were large enough to minimize body movement, the arms didn't swing. They didn't need to. But when the rotational friction at the foot was constrained to zero, the arms swung naturally in compensation. This was true for two different styles of walking—static (a kind of slow stagger where the center of mass is always over one foot or the other or both) and dynamic (a more realistic style at a normal human pace).

To Park, these results suggest that arm swinging helps us maintain our balance on slippery surfaces because it compensates for the absent rotational friction. In addition, it provides greater comfort, since the foot and consequently all the leg joints do less work.

"This paper has elucidated the relationship between arm swing and the support moment at the foot," comments **Marcus Pandy, PhD**, chair of mechanical and biomedical engineering at the University of Melbourne, Australia. More work remains to be done, though, to understand the relationship between the foot's role and "energy consumption during gait." Pandy also notes that "it would be interesting to see how the joint torques predicted by the model compare with those obtained from experiments when humans walk at their preferred normal speeds."

In the future, Park would like to explore whether arm swinging affects the speed of movement. Eventually, such work might provide more evidence that there is a good reason to swing.
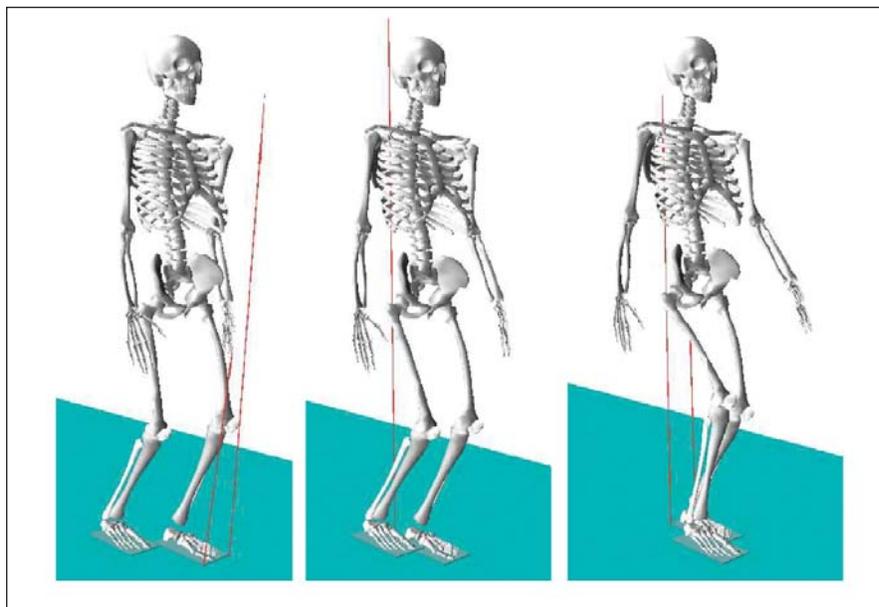—*By Meredith A. Kunz*

A simulation of human walking with zero friction at the foot generates natural arm swing motion. Courtesy of Jaeheung Park. Reprinted from Journal of Biomechanics 41: 1417-1426, 2008 with permission from Elsevier.

RNA Takes Shape

RNA is not just a single-stranded template. Like proteins, many RNA molecules can fold into three-dimensional structures that catalyze reactions and regulate gene expression. Predicting this structure, though, remains an open challenge. Scientists at the University of Montreal have devised a novel way to attack the problem, which they describe in the March 6 issue of *Nature*.

"Our approach is to generate a more complete RNA secondary structure and from there go to three dimensions directly. Whereas before going to 3-D from secondary structure was impossible," says **François Major, PhD**, professor of computer science and operations research, who developed the method with graduate student **Marc Parisien**.

RNA nucleotides bind with each other to form secondary structures such as hairpins (a stem with a loop) and helices. Though most nucleotides pair according to Watson-Crick or wobble rules (C-G, A-U, and G-U), a small number (about 15 percent of nucleotides in hairpins, for example) form alternate pairings—such as A-C or a G-U-A base triple (where the bases meet in different orientations). Previous programs have fallen short of predicting these "non-canonical" pairings that are the key to

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3-D structure and indeed often drive the most interesting geometries such as loops, bulges, and twists.

To better predict non-canonical pairings, Major and Parisien identified 19 regular, repeated small motifs (mostly 3 to 5 nucleotides) in solved RNA structures. They call these the RNA structural alphabet or "nucleotide cyclic motifs" (NCMs). The most common "letter" (or NCM) consists of two Watson-Crick base pairs stacked on top of each other; a bunch of these together form a basic helix. But many of the other NCMs are defined by non-Watson-Crick base pairs. One example is a four-nucleotide loop with a G-A pair at the bottom.

To determine the 3-D structure of a given RNA primary sequence, Major and Parisien feed it through two programs: MC-Fold and MC-Sym. MC-Fold enumerates all possible base pairings (including non-canonicals) and all possible arrangements of NCMs. It then picks the most probable arrangement based on statistical data from solved RNA structures. Next, MC-Sym translates the NCMs directly into 3-D structures. The pipeline is available as a web service (http://www.major.iric.ca/MC-Pipeline/). Currently, accuracy is limited to sequences of fewer than 75 base pairs—unless experimental or multiple-sequence data are incorporated into the program, Major says.

As a test case, Major and Parisien folded several precursor microRNAs (with previously unknown structures). Such molecules would be expected to share a common structural element for binding to the enzyme Dicer, which processes them into functional microRNAs. The result: despite different primary sequences as well as non-canonical base pairs and bulges, the pre-microRNAs all folded into double helices.

"That's a pretty powerful result," comments **Philip Bevilacqua, PhD**, professor of chemistry at Penn State University. "I think this method is going to be of practical benefit to the RNA community," he says. "This has the potential for enormous impact, and hopefully it will get fulfilled."
—*By Kristin Sainani, PhD*
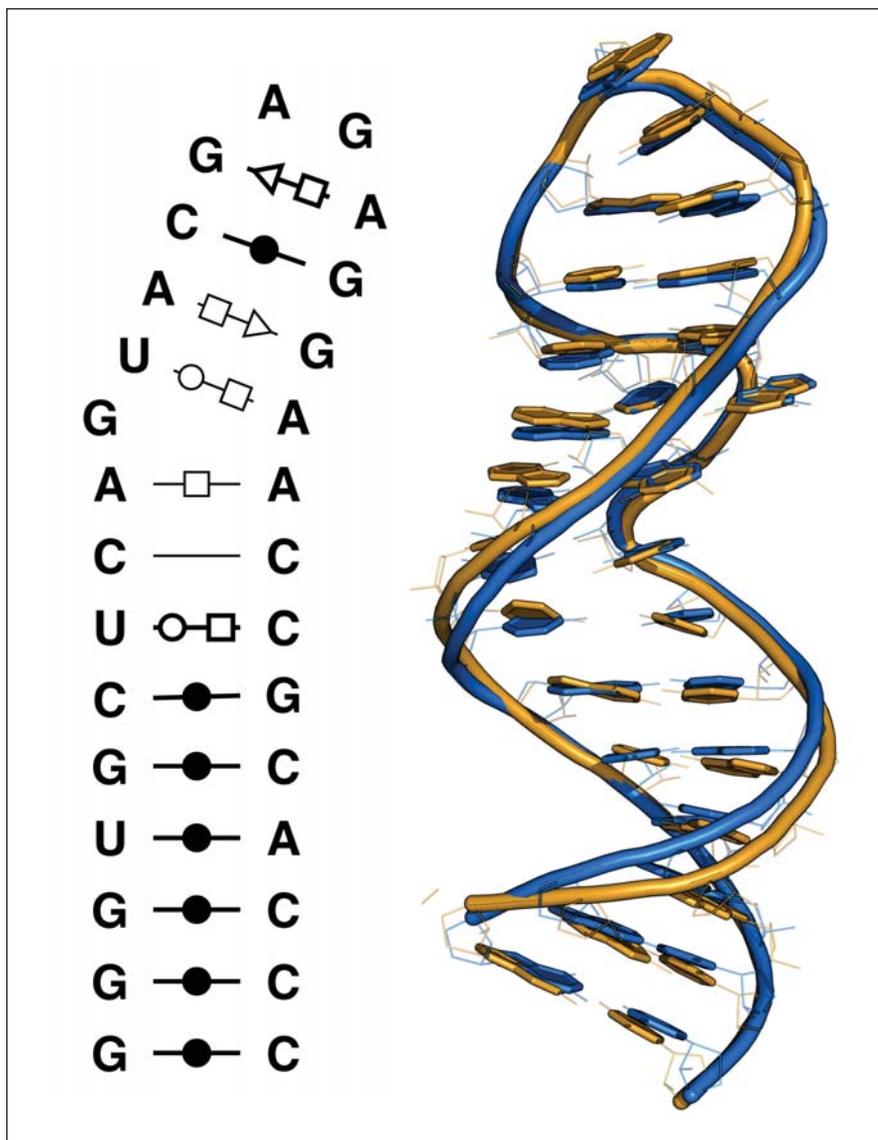
Predicted 2D and 3D structures of an RNA loop (Sarcin/Ricin loop from rat ribosomal RNA). Left: Watson-Crick (black dots) and non-Watson-Crick base pairs predicted by MC-Fold. Right: predicted 3D structure (blue) superimposed on the experimentally determined structure (gold). Courtesy of Francois Major and Mark Parisien.

Trojan Peptide

A powerful snippet of protein called the Tat peptide ferries itself across cell membranes dragging just about anything it's attached to along with it. How it accomplishes this feat has been a puzzle for a decade. Now, computational simulations offer a detailed picture of how the string of eleven amino acids cajoles the membrane's lipid bilayer into doing most of the work.

"I was expecting that the peptide would act like a snake going through a hole," says **Angel Garcia, PhD**, professor of biocomputation and bioinformatics at Rensselaer Polytechnic Institute, who helped design the simulations. Yet his laboratory's simulations suggest that instead of the snake doing all the work, it is as if the ground makes space for the snake to pass. "I wasn't expecting the lipids to change so drastically," he adds.

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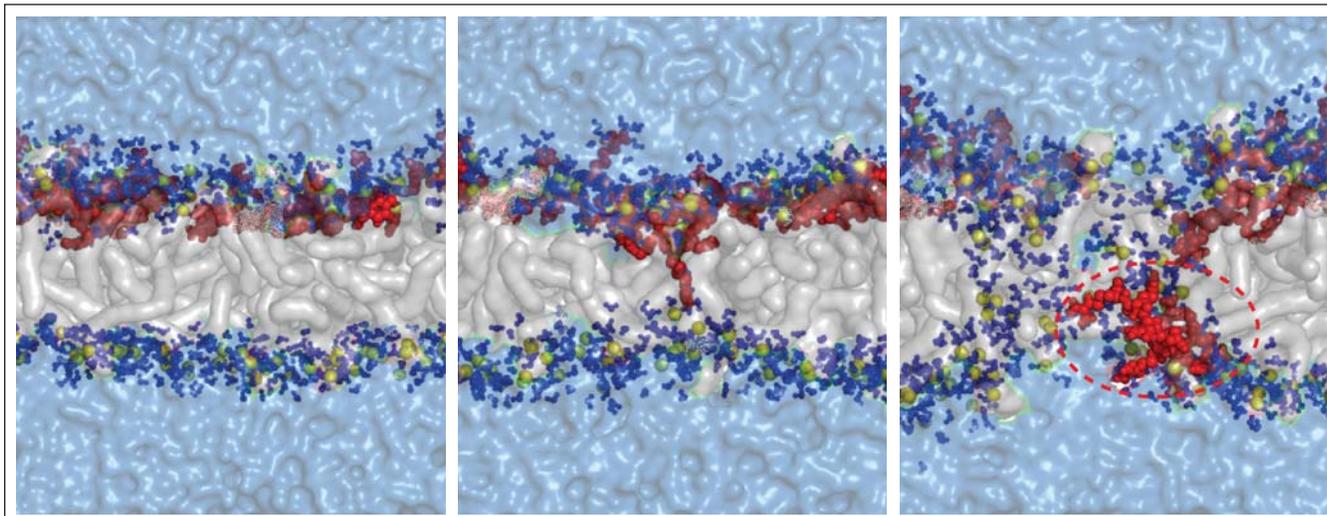
At left, four Tat peptides (red) cluster on one side of a lipid bilayer (white) attracted to the phosphate groups (yellow). As the Tat peptide reaches toward phosphate groups on the opposite side (middle), the bilayer thins enough for a chain of water molecules (blue) and the peptide to pass through the membrane (right). Courtesy of Angel Garcia. Reprinted from Proceedings of the National Academy of Sciences *104:52 (2007).*

"Once you see it, of course, it could not be any other way." The work was published in *Proceedings of the National Academy of Sciences* in December 2007.

The Tat peptide, discovered on an HIV protein, is part of a potent group of cell-penetrating peptides sometimes called Trojan horse peptides. They haul drugs, proteins or DNA right across the lipid bilayer and into the cell. The myriad uses of such peptides in both therapy and research are not hard to imagine. But how these highly charged, water-loving bits of protein so readily cross the waterless middle of the lipid bilayer has evaded answer for years.

Garcia and postdoctoral fellow **Henry Herce, PhD**, decided to apply the power of a new computer center at RPI to conduct molecular dynamics simulations of the Tat peptide as it approaches and crosses a lipid bilayer.

Over and over again, the simulations reveal how the peptide induces a change in the bilayer. Because six of the eleven amino acids in Tat are arginine, a relatively large, positively charged amino acid, researchers knew that Tat would be strongly attracted to the lipid bilayer with its blanket of negatively charged phosphates. But Garcia did not expect that phosphates on both sides of the bilayer—not just on Tat's side—would align to help neutralize Tat's charge. The more peptides added to the mix, the greater the influence on the opposite side of the bilayer. As the arginine side chains and distant phosphate groups move toward each other, the bilayer thins until it creates a hole lined with phosphate groups, letting a small chain of water and the peptide pass through.

"The idea that the bilayer is 'thinned,' thereby allowing the cationic TAT to touch anionic phosphate head groups on both sides of the membrane was utterly unexpected," says **Steven Dowdy, PhD**, a Howard Hughes investigator and professor of cellular and molecular medicine at the University of California, San Diego. Dowdy says the information from Garcia's computational work will inspire experimental testing of the mechanism. And, he says, it could be very helpful in designing enhanced peptides with increased potential to deliver drugs or DNA where researchers want them.

—By Louisa Dalton

Window into Microbial Behavior

We know they are there, but most microbial denizens of deep oceans, sea floor vents, even our own intestines, remain a mystery. Because most microbes won't grow in the lab, researchers have few clues to their communal activities.

With better gene sequencing and computational ability, researchers now sample genes from whole communities to assemble the "metagenome"—a picture of the genes driving metabolic processes important to growth and survival in a given environment.

In a new study, researchers found remarkable diversity in how microbes function in each of nine distinct biomes. Indeed the bacterial and viral genomes from each biome had distinguishing metabolic profiles. And viral genomes—which researchers expected would be similar across environments—were just as different as the bacteria.

It turns out that there's a surprisingly extensive genetics arms race going on between bacteria and the viruses (called phages) that infect them, says **Rob Edwards, PhD**, assistant professor in the Computational Sciences Research Center at San Diego State University. Viruses are actively shuffling their host bacteria's DNA. "We didn't know (just) how much DNA the viruses move around," Edwards says. In fact, it happens so often that, he believes, the viruses likely profit from moving pieces of DNA that are beneficial to the bacteria.

Edwards and his collaborators from San Diego State University, Argonne National Laboratory and around the world reached these conclusions by comparing nearly 15 million sequences from

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45 microbial communities, including 42 viral genomes, as reported in *Nature* on April 3, 2008. It's easy and relatively inexpensive to generate a DNA sequence these days, Edwards says, "What is not so easy is to figure out what it actually means."

Thanks to the SEED database (www.theseed.org), developed in collaboration with researchers at Argonne Labs and the Fellowship for Interpretation of Genomes, which annotates or assigns known function to gene locations, scientists can upload gene sequence data and seek a pattern of metabolic activities that exist in their samples. They can thereby begin to compile the collective activities of a given community, be it a coral reef, a mine shaft, or a person's bronchi.

This sort of work will definitely help researchers understand and harness the functions of bacteria, says **Eric Delwart, PhD**, a virologist at the Blood Systems Research Institute and the department of Laboratory Medicine at the University of California, San Francisco.

"Bacterial genomes are scrambled and slapped together by viruses. The core functions probably cannot be exchanged, but peripheral functions can be passed around," he says, in a process unique to bacteria that likely speeds up their rate of evolution.

Such gene swapping may also yield therapeutic insight. A lot of diseases, such as atherosclerosis and stomach cancer, have "a very strong microbial component," Edwards says. "We are working with the NIH to get at the bioinformatics of this."
—By Roberta Friedman, PhD

How the Zebrafish Gets its Stripes (or Spots)

Normal zebrafish have stripes, but mutant forms may display spots, blotches, or labyrinthine patterns. It's a scenario that Rudyard Kipling might turn into a wonderful "just-so" story. But a


Normal and leo mutant zebrafish (left) and their corresponding normal and abnormal simulated patterns (right). Courtesy of Troy Shinbrot and David Parichy. Reprinted from **Developmental Biology, 315, Caicedo-Carvajal, CE; Shinbrot, T, In Silico Zebrafish Pattern Formation, 397–403 (2008), with permission from Elsevier.**

more scientific explanation comes from a new computer model that can replicate the diverse ways that pigmented cells organize themselves on zebrafish skin. The results may help scientists gain a better understanding of development in general, helping explain how myriads of cells turn into tissues, organs, and entire organisms.

"Our aim here is not to build better zebrafish," says **Troy Shinbrot, PhD**, who developed the model along with his graduate student, **Carlos Caicedo-Carvajal**. "We want to understand how tissues and organs develop and how cells migrate, survive, and form the shapes that govern function." The work was published in *Developmental Biology* in January 2008.

According to Alan Turing's theory from the 1950s, pigmented cells arrange themselves into patterns under the guidance of chemical agents. More recent studies of zebrafish stripe formation suggest that mechanical interactions between cells—how strongly they push or pull one another—could also play a vital role. To test the latter hypothesis, Shinbrot and Caicedo-Carvajal developed a simplified energy-minimization model of cells of two different colors interacting within a rectangular region.

Using different combinations of values for the forces between like (homotypic) and unlike (heterotypic) cells, the researchers generated a range of possible patterns. "To get stripes, we need both heterotypic attraction and a delicately balanced homotypic repulsion," says
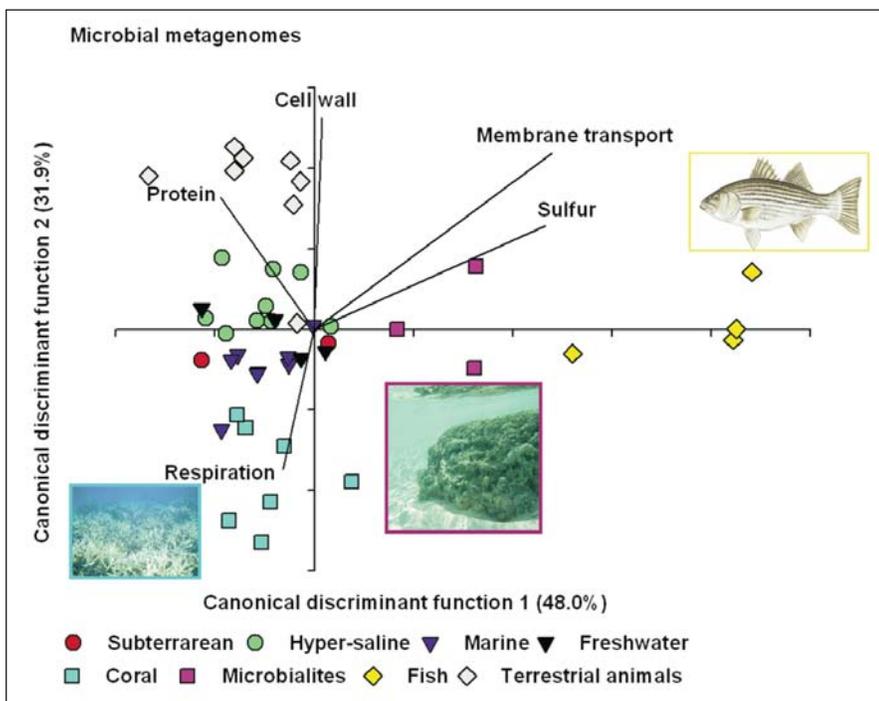
The survival techniques of bacteria in nine different biomes (represented by different colored symbols) can be distinguished based on the prevalence of various metabolic gene subsystems such as respiration, membrane transport, virulence, or sulphur metabolism. The length of the lines represents the degree of influence of a metabolic process. Courtesy of Elizabeth Dinsdale. Reprinted by permission from Macmillan Publishers Ltd: **Nature, 452, 629 - 632 (12 Mar 2008). Coral and microbialite photos by F. Rohwer.**
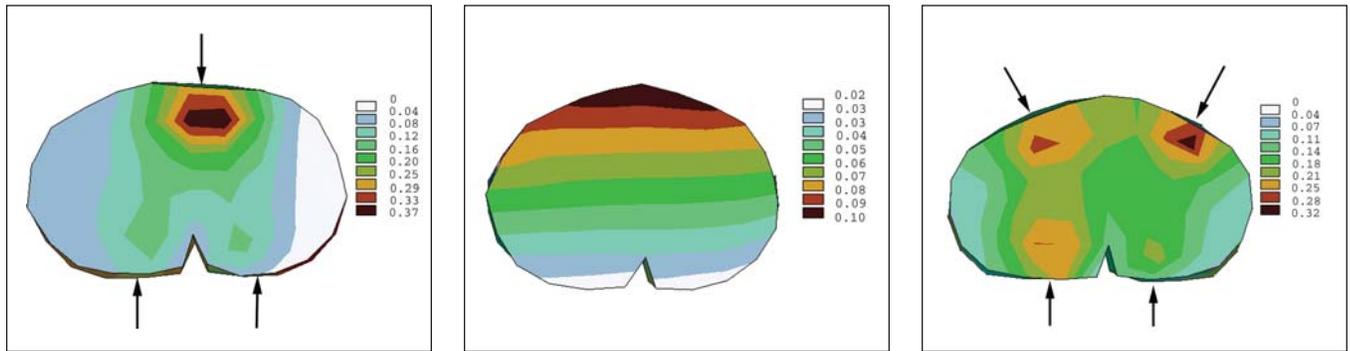
These cross-sections of a simulated spinal cord show the different deformation patterns induced when the cord is subjected to a transverse contusion injury (left), a distraction injury (center) and a dislocation injury (right). Courtesy of Carolyn Greaves. Reprinted from Greaves, C, Gadala, M; Oxland, T, A Three-Dimensional Finite Element Model of the Cervical Spine with Spinal Cord: An Investigation of Three Injury Mechanisms, **Journal of Biomechanical Engineering** *36:396 (2008) with kind permission of Springer Science and Business Media.*

Shinbrot. If these conditions were not met, the simulations showed that spotted, striated, labyrinthine, and other non-striped patterns developed; in particular, when all the inter-cellular forces were attractive, only spots formed. The researchers showed that some of these abnormal patterns resemble those observed on certain mutant zebrafish varieties with defective pigment pathways.

This *in silico* approach could be applied to a broad range of problems in cellular development, says Shinbrot. The researchers are now using it to help oncologists compare four different patterns of abnormal tissue commonly seen in early breast cancer tumors.

"The authors have done a nice job of showing how you can produce a whole repertoire of patterns simply by tuning the strengths of attractive and repulsive cell interactions," says **Ed Munro, PhD**, a computational cell biologist at the University of Washington in Seattle. However, Munro cautions that the results obtained using the authors' simplified model need further biological validation. "By demonstrating one way in which cells can make patterns, you haven't shown that's how embryos do it," he notes.

—*Chandra Shekhar*

Modeling the Spine, Cord and All

When the bones and discs of the spinal column are broken, crushed, or displaced, the spinal cord itself may be devastatingly damaged. Now, a new computer model suggests that the manner in which the injury occurred may affect the spinal cord in distinct and significant ways.

This work could have a wide-reaching impact on spinal treatment, says **Thomas Oxland, PhD**, professor of orthopaedics and mechanical engineering at the International Collaboration on Repair Discoveries (ICORD) Centre at the University of British Columbia. If cord injuries could be subclassified by type, it is possible that physicians may be able to treat them differently. Oxland was lead author of the work, published in *Annals of Biomedical Engineering* in March 2008.

Before modeling the human spine, Oxland's team, which included his master's student **Carolyn Greaves**, and **Mohamed Gadala, PhD**, a professor of mechanical engineering, had already begun animal studies to examine the relationship between the type of spinal column injury and the strain on the cord. But they wanted to compare their animal data to the human spine. Because it's impossible to use human experimental models, the group simulated the spine and the spinal cord using data from the Visible Human project.

Like others who have modeled the spine, Oxland and his colleagues created a finite element model of the human cervical (neck) spine. They then simulated injury to it by applying engineering torques, not unlike those used to study the strain on a bridge. What's new here

is that they observed the effect of different types of injuries on the spinal cord itself. The result: distinct patterns of strain and deformation depending on whether the spine suffered a burst fracture, a dislocation, or a stretching injury. The work stopped short of examining actual cord damage but, Oxland says, "one would expect that [these mechanisms] would produce very different patterns of damage in the cord."

Oxland acknowledges that their now-static model cannot yet capture the dynamic forces at work when a real-life injury happens, often in a fraction of a second. His team is working on introducing more variables and lifelike properties now. He also plans to match up the simulation results with his lab's animal experiment data to better understand cord damage.

David Shreiber, PhD, an assistant professor of biomedical engineering at Rutgers University, thinks this model will help advance the field—one that still lags behind brain injury research. "It's significant because it's the foundation of more work on injury to the cord," says Shreiber. The model is flexible enough that it can be used to understand many types of injuries. "The nice thing about this computational system is that you can apply the loading conditions however you want—you can look at twisting, at pressure applied internally, and other cases of spinal injury," he adds.

—*Meredith A. Kunz* □