

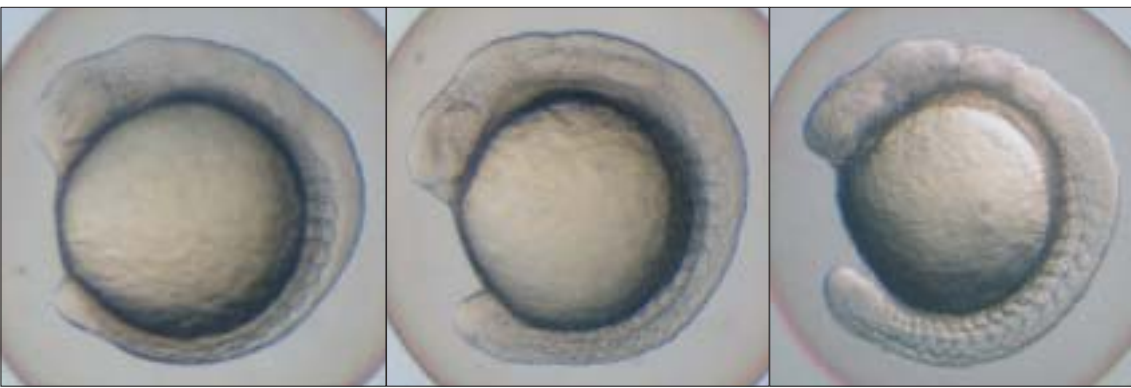


# MODELS WITH GILLS

ZEBRA FISH DO SWIMMINGLY | BY DOTTIE HORN

**A**fter the first flickers of light, the courtship begins. Two zebra fish swim in still water. The male nudges the female. She emits her eggs, a few at a time, each visible to the naked eye. The male follows the female, exuding invisible sperm. In the wild, the fish usually turn around and eat their would-be progeny. In the lab, the descending eggs pass through a screen to safety.

It is a ritual soon to become commonplace at the School of Medicine. And, to many scientists, all those new zebra fish may be the best thing since sliced fruit flies.



**The zebra fish's transparent and quickly developing embryo has caught scientists' attention. ABOVE: Every 40 minutes or so, these zebra fish embryos grow another somite—which later develops into a single vertebra and its associated muscle. Look closely in the images and you can count the individual somites. In the first picture, about 13 hours after fertilization of the egg, the embryo has 10 somites. About two-and-a-half hours later, it has 14; in just another two-and-a-half hours, it has 18. The embryo is curled around a large round yolk sac, which contains enough food for its first five days of life.**

Nathan Bahary and Neil Hukriede, assistant professors of molecular genetics and biochemistry, have joined the faculty at the University of Pittsburgh and will oversee Pitt's first large-scale zebra fish facility. (The researchers recently completed postdocs at Harvard University and the National Institutes of Health, respectively.) When the facility's construction is complete, a handful of rooms located in the Biomedical Science Tower will house about 2,500 tanks and 35,000 fish.

The zebra fish, a relatively new animal model first used in the mid-1980s, has become increasingly popular in laboratories. In 2005, it will become the third vertebrate to have its genome completely sequenced, joining the human and the mouse. Because it's a vertebrate, the fish shares more genetic similarities with us than the fruit fly, a longtime reigning lab model.

Why are scientists, particularly those

studying how organisms develop, so taken with the zebra fish? In fish, the embryo grows outside the mother's body—and in zebra fish, the embryo and its egg sac are transparent. Those accessible, optically clear embryos are a boon. "You can literally watch the heart start beating or the brain start to grow," says Bahary. Zebra fish also develop quickly. In the first 24 hours of life, a single cell becomes an embryo with a tail, heart, blood vessels, circulating blood, and a brain. By its third day, the fish has a complete digestive system and can eat—a level of development that takes 30 days in the mouse. Another advantage: You can house about 50,000 zebra fish in the space it would take to accommodate a few hundred mice.

Perhaps most importantly, zebra fish allow scientists to do research that would be difficult in some species. In one study, the male

zebra fish is soaked in ethylnitrosourea before it is bred; the chemical induces mutations in its DNA. By breeding its offspring with each other, scientists generate slews of fish with random single mutations. In one, the brain may be malformed; in another, the heart. "You can find many different mutations involving pretty much any organ system that you want," says Bahary.

Some of the embryos are so deformed they will survive only a day or two, but while they live, researchers can watch as development goes awry. Scientists can take DNA from a mutated fish—say, one that has an abnormal liver—and find the gene responsible for the malformation. They then can look to see if the same gene is found in humans—and it usually is. The next step: Find out if the gene is mutated in people who have a disease affecting the organ system in question. Through such tests, zebra fish are helping researchers identify genes involved in human disease.

Hukriede uses zebra fish to study how kidneys develop. Bahary was drawn to the model in the hope that new cancer treatments might emerge from studying it. The oncologist examines the development of blood and the gut. He has a mutant zebra fish that is bloodless and is working to clone the gene, named *cloche*, responsible for the mutation.

"If we can figure out how organs normally form," says Bahary, "we'll be able to then find targets for therapies in humans." ■

# QUIET TIME

TUCKING IN DNA | BY ERICA LLOYD

**O**n one of Oregon's many damp January days, Sanford Leuba found himself under his Chevette, not an odd place to find him during those graduate student years. Leuba was in the midst of changing his clutch plate and at a particularly tricky point. He lay on his back with the transmission resting on his chest, attempting to slide a piece back into a pilot bearing on the flywheel, but he couldn't feel where the pilot bearing was. After hours in that position,

A single chromatin fiber, seen through atomic force microscopy



searching millimeter by millimeter of a Chevrolet void—*slip*, the piece fell into place. Now, in Leuba's heart of hearts he knew a few things: 1) If he dared to do anything but attach the needed bolt right then and there, his chances of locating the pilot bearing again at that point were infinitesimal. 2) He really should be spending more time on his PhD and less under cars. 3) If he could change a clutch plate in a Chevy, he could do just about anything.

He handily finished the clutch-plate job and, eventually, his PhD in biochemistry and biophysics at Oregon State University (OSU) in Corvallis. Thereafter, Leuba would become known for some highly sophisticated tinkering, most notably, his dexterity with new tools that are teasing apart our understanding of biology at the nanometer level.

In popular media, DNA is represented as a telltale double-helix figure, standing alone. But if you were to look at the molecule in a human cell, you would find it wrapped around thousands of histones and other proteins that coil and bind the 2-meter-long strand so that it fits into the cell nucleus. "Our DNA is not naked," Leuba asserts.

This complex that surrounds all nonbacterial DNA—known as chromatin—is more than packaging. It plays a role in DNA transcription, replication, and repair.

"We're realizing that all of the biological processes that deal with DNA have to occur within the context of chromatin," says Leuba, now an assistant professor of cell biology and physiology at the University of Pittsburgh. One of chromatin's key jobs seems to be keep-

ing DNA "quiet" by keeping the molecule tucked in, so to speak. "Most genes in your cells are turned off," says Leuba. "You want that. Aberrant processes are typically cancerous."

Chromatin activity, or lack thereof, already has been linked to ovarian, cervical, and colon cancers, to leukemia—the list is broad. In fact, if you type "chromatin and cancer" into the National Library of Medicine's online literature search engine, you'll get more than 5,000 hits.

Still, Leuba points out, "Little is known about how [DNA] processes occur within a chromatin template." For example, modifications to histones are likely to play important roles; but researchers, for the most part, tend to study chromatin by looking at batches of DNA, rather than just one molecule at a time. That tells you little about what an individual histone might be up to. Such approaches aren't very satisfying to Leuba. Ken van Holde, Leuba's mentor at OSU and author of *Chromatin* (known to some as "The Green Bible"), remembers his student being an "instigator," pushing the lab to apply atomic force microscopy, a single molecule technology, to chromatin studies.

"It became clear that Sanford was comfortable with new technologies; they didn't intimidate him in any way," says van Holde. ("I have no fear," admits Leuba.) The esteemed OSU professor says he himself might have shied away from trying the new tool at first; instead, his group soon became known for showing the world that one can, indeed, examine chromatin molecule by molecule.

Two years ago, as a National Cancer Institute scholar, Leuba went on to unravel

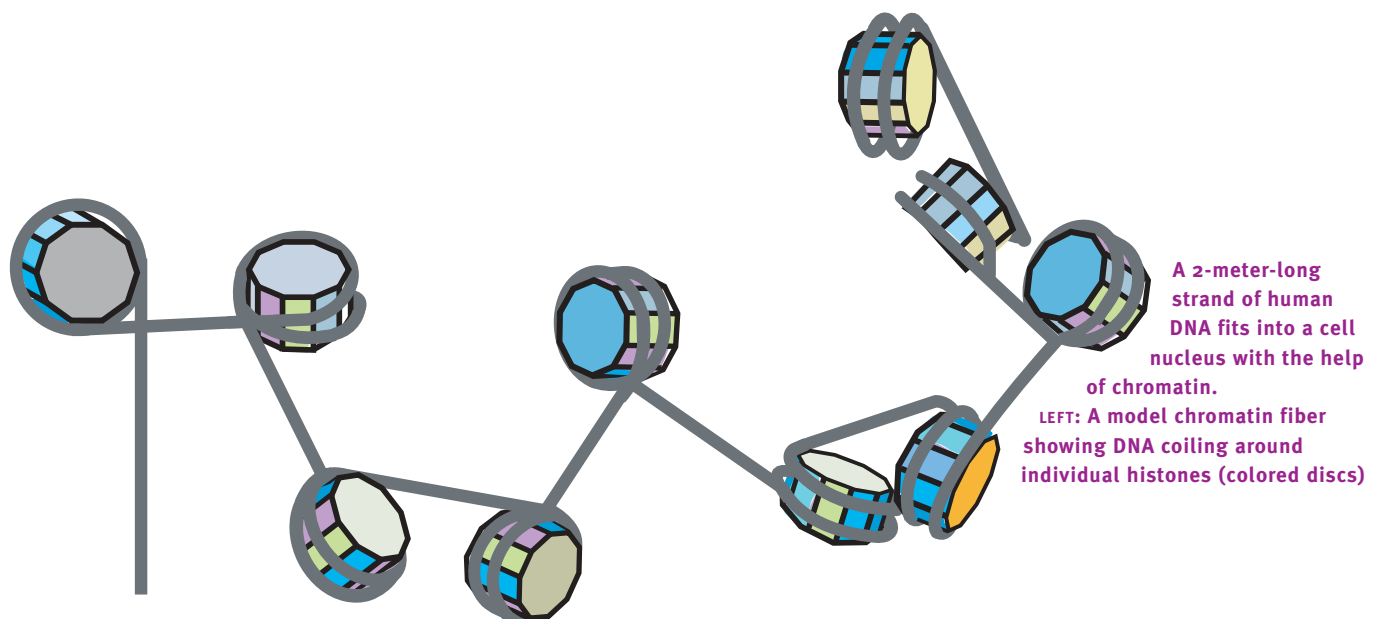
chromatin. Literally.

With Dutch collaborators, he tethered a tiny polystyrene bead to each end of a strand of bare DNA and then bathed the strand in a flow of frog-egg extract. By holding one bead in place (with suction from a pipette), the group could record the change in the length of the strand as the flow passed through. What they saw made sense: The strand shortened—the DNA appeared to bind to proteins and further coil and compress itself into a size that could squeeze into a cell nucleus. They had, in effect, assembled a chromatin fiber.

After that, they held the free-floating bead with "laser tweezers," which would allow them to measure any change in force precisely. Then they pulled on the other bead, very carefully. The amount of force needed to unravel the fiber increased and gave slack suddenly, in increments of about 65 nanometers. That was a magic number to Leuba and associates. Why? Nucleosomes, which can be thought of as popcorn balls of histones (eight of the proteins bunched together make up a nucleosome) are understood to occur about every 200 DNA base pairs, which translates to 60-some nanometers. Leuba and colleagues had managed to tease apart a key structural component of chromatin, at the same time recording the biological forces that held it in place.

"It was beyond any result we'd hoped to see," Leuba says, happily. ■

**FOR MORE INFORMATION:**  
[cbpmedia.cbp.pitt.edu/leuba/leuba.html](http://cbpmedia.cbp.pitt.edu/leuba/leuba.html)



**A 2-meter-long strand of human DNA fits into a cell nucleus with the help of chromatin.**  
**LEFT: A model chromatin fiber showing DNA coiling around individual histones (colored discs)**

M. KARIMOV AND C. H. ROBERT, RENDERED BY LOTENERO



# CRASH TESTS

MANY HIGH SCHOOL ATHLETES SUFFER CONCUSSIONS —  
WHAT'S THE LONG-TERM DAMAGE? | BY TRINA WOOD

**C**oaches and trainers rush across the football field toward the teenager lying motionless on the grass. Seconds before, he had been flying through the air after a leather ball. Then came the big crash into another body jostling for the coveted prize. With no sign of consciousness from the high-school athlete, a coach calls for the medical emergency helicopter.

The young man's brain had rocked back and forth inside his skull—he'd suffered a concussion. He was in danger of experiencing confusion, disorientation, dizziness, and amnesia. If his case proved to be particularly severe, he might end up with brain swelling or cell or blood vessel damage. His crash could even prove fatal.

Approximately 10 percent of high-school athletes in contact sports such as football and hockey experience concussions each season, says Mark Lovell, a PhD, assistant professor of orthopaedic surgery at the University of Pittsburgh, and director of the UPMC Center for Sports Medicine Concussion Program.

"Teens are the largest group of at-risk athletes," Lovell says. "Given that their brains are still developing, we think it is particularly

important to protect their health."

About five years ago, Lovell, then in Detroit, began collaborating with Joseph Maroon, Pitt clinical professor of neurological surgery, to develop a computerized version of ImPACT (Immediate Post-concussion Assessment and Cognitive Testing)—a test used to evaluate concussions. The new ImPACT is a 25-minute assessment that measures brain processing and speed, memory, and visual motor skills.

Seventy high schools in the Pittsburgh area and more than 100 college and professional sports teams now use the program to monitor their players. At the beginning of the season, team athletic trainers and physicians collect and store baseline ImPACT data on athletes' neurocognitive functions. If an athlete experiences a concussion during the season, he or she can be retested to help physicians assess a concussion's severity and determine when and if an athlete should return to contact sports.

Animal studies conducted at the University of California–Los Angeles have shown that, until the brain returns to normal following a concussion, there is an

increased risk and vulnerability to another injury.

"We want to make sure we're not putting people back into play when they're in that vulnerable period," says Lovell.

Pitt recently received \$2.8 million in funding from the National Institutes of Health to conduct a five-year study using functional MRI (fMRI) on 250 high school athletes from western Pennsylvania schools. Study participants are athletes who've taken a baseline ImPACT test and then suffer a concussion during the season. If an ImPACT conducted within 72 hours of the injury indicates decreased neurocognitive function, the teen is sent to Pitt's Center for Sports Medicine for an fMRI.

Michael Collins, an instructor of orthopaedic surgery and PhD psychologist, explains that during the fMRI, researchers re-administer part of the ImPACT. Each question measures an aspect of brain function that is vulnerable to concussion. During the fMRI, researchers can spot irregularities in patterns of brain activation.

"When we use specific parts of our brain—for example, to talk, remember, or to think—there are measurable changes in brain chemistry and blood flow," Lovell says. "Using the fMRI, we can measure those changes and link the athlete's performance on ImPACT to what is actually happening in the brain." Preliminary results show increased metabolic activity in the cerebellum in those suffering from a concussion (even if the impact was to another brain area).

Are males or females more vulnerable to concussions? Do concussions make a person more vulnerable to dementia later in life? Those who lose consciousness or have amnesia due to a concussion—is their outcome likely to be worse? Lovell and Collins will use the data collected on local high-school athletes as they consider these and other questions. The answers, they hope, will translate into better care for athletes—especially teens.

Fortunately, the high-school football player who was flown off the field to an emergency room demonstrated no neurocognitive damage 10 days after his injury. This was his first concussion, and, after a period of rest, he was cleared to return to the field. ■