chronicle

A DNA molecule's intricate twists and turns provide unique spots for proteins to bind. New research has shown that proteins (blue- and red-knobbed structures) prefer binding to areas with negative electrical potentials (red), most often found in the narrow groove between turns of the DNA helix (see page 40).

Honig Lab

Histones Wreaking Havoc

A CLOSE LOOK AT INFECTED BLOOD SAMPLES REVEALS HOW SEPSIS SPIRALS OUT OF CONTROL.

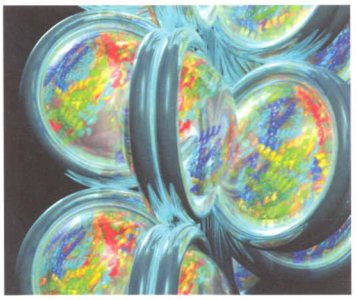
The proteins that keep DNA wound tightly inside a cell's nucleus—called histones—have no place outside the cell. New research suggests that when they land in the bloodstream, they encourage sepsis, the potentially deadly response of the immune system to severe infection. Destabilizing histones that escape the cell can halt the spiral of events that make sepsis lethal.

HHMI investigator Charles T. Esmon's lab group at the Oklahoma Medical Research Foundation previously showed that a compound called activated protein C (APC) could block sepsis. Although a commercial drug, Xigris, was developed from APC, its mechanism remained unclear, and it didn't work in all cases. Esmon was determined to further unravel the molecular basis of sepsis.

The idea that histones might play a role in sepsis came serendipitously. Jun Xu, a postdoctoral fellow in Esmon's lab noticed histones in cultures where macrophages—a type of immune cell—had become inflamed and cleaved and their toxic activities neutralized by APC.

"People had seen histones in the blood before," says Esmon, "but assumed they leaked out of cells as a side effect of the major damage caused by sepsis. No one ever guessed they had a more central role."

To determine whether the chopped up histones were more directly related to sepsis, the researchers exposed blood vessel cells—normally damaged during sepsis—to histones and to histone fragments. The intact histones killed cells while the histone fragments did not.



Normally, histones act as spools for DNA to wrap around, as shown in this artist's rendering.

Esmon's team also looked at mice, baboons, and humans with sepsis—they all had free-floating histones in their blood. When the researchers gave a histone-blocking protein to septic mice, which were expected to die, many of the mice survived.

The data, published in the November 2009 issue of *Nature Medicine*, suggest a new theory on sepsis: Initial cell damage, from widespread inflammation due to an infection, lets histones leak into the bloodstream. These histones kill more cells, which release more histones, in a vicious cycle. APC, scientists now know, cleaves those histones to stop sepsis. The severe side effects of APC, however, make it a last resort drug. Other histone blockers, which may cause fewer side effects, can now be tested on sepsis. ■ –SARAH C.P. WILLIAMS

IN BRIEF

TAKING SIDES

It's a myth that some people are "right-brained" and others "left-brained," but it is true that the brain divvies up jobs between sides. An imaging technique developed by HHMI investigator Randy L. Buckner reveals just how complex this division is.

Researchers use functional magnetic resonance imaging (fMRI) to link brain regions to certain tasks. fMRI measures blood flow—increased blood flow in an area indicates neuron activity. In a typical fMRI experiment, researchers ask a participant to perform a task—like watching images or memorizing a list—and then observe what brain area becomes active.

But Buckner's lab group, at Harvard University, wanted to know how the brain divides its baseline activity, unrelated to any task. So they asked each participant to lie still and stare straight ahead while they monitored the brain's constant chatter. The scientists tracked areas on both sides of the brain that spontaneously fired at the same time—indicating that they were doing matching or complementary jobs—and measured which side showed greater activity.

When the neurons that fired on each side were in areas known to be linked to language, the activity tended to be stronger on the left side; neurons in regions linked to vision and spatial awareness were stronger on the right. But not in everyone. In some people, the pattern was reversed, and in others the sides were less lopsided.

Now that Buckner has shown the links between sides of the brain can be observed in a resting brain, as reported in the *Proceedings of the National Academy of Sciences* on December 1, 2009, he hopes to investigate further how genes control the division of labor between the two sides of the brain, a question relevant to developmental disorders such as autism and schizophrenia.

TARGETING TICK SALIVA

Blocking a protein in tick saliva reduces the risk of a mouse becoming infected with Lyme disease, researchers have found.

HHMI investigator Erol Fikrig at Yale University discovered the protein, Salp15, in 2005. He found that the Lyme disease pathogen, *Borrelia burgdorferi*, ramps up the tick's production of Salp15. The bacterium then coats itself in the protein, hiding from the tick's immune system. Fikrig wondered whether a protein that blocked Salp15 could block Lyme disease.

His team injected mice with a Salp15 antiserum and then a day later with Salp15-coated *B. burgdorferi*, as it would be if the bacteria were transmitted from a tick. Three weeks later, 40 percent of these mice remained free of Lyme disease. All the control mice, which received an inactive antiserum before the bacteria, contracted the disease.

Previously developed Lyme disease vaccines have been dropped from the market. Fikrig's team tested whether combining those vaccines with Salp15 would increase their efficiency. The combination was a winner—only 25 percent of mice receiving both compounds showed signs of Lyme disease. The results appear in the November 19, 2009, issue of *Cell Host & Microbe*.

DOUBLE HELIX, REVISED

A half century after Francis Crick and James Watson discovered the famous double-helix shape of DNA, scientists are only beginning to fully understand its subtleties. New research by HHMI investigator Barry Honig has shown that slight variations in the helix shape allow DNA-binding proteins to differentiate between regions.

Honig, at Columbia University, became interested in the nuances of DNA shape

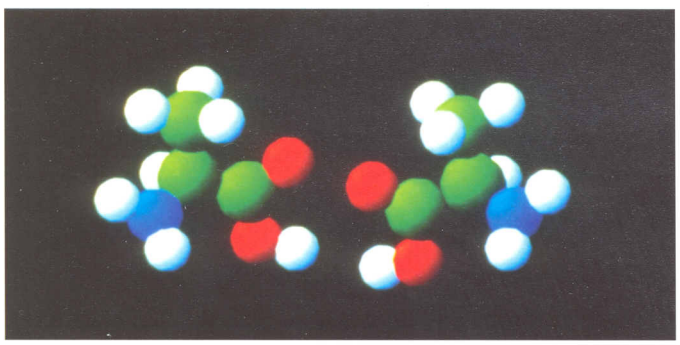
Laguna Design / Photo Researchers, Inc.

Righty, Lefty

AN UNUSUAL TYPE OF AMINO ACID ACTS AS A GROWTH SIGNAL IN BACTERIA.

Amino acids—the building blocks of proteins—are chiral molecules, which means they can exist in two mirror forms. While L-forms of amino acids are predominantly found in nature, the D-forms are less abundant and little is known about their biological function. But new research shows that the few D-amino acid outliers play a vital role in regulating cell wall growth in bacteria.

Matthew K. Waldor, an HHMI investigator at Brigham and Women's Hospital, was studying how *Vibrio cholerae*, the bacterium that causes cholera, gets its characteristic rod shape. His lab identified a mutant that became spherical when cultured overnight. Waldor's team suspected that something was accumulating in the culture that affected the mutant bacterium's shape. They analyzed the chemicals floating in the soup around the bacteria and found a plethora of right-handed, or D-amino acids.



The amino acid alanine in its L- and D-forms, mirror images of one another.

"This was really amazing," says Waldor. "No one even knew that bacteria make these types of D-amino acids, and not only were they there, they were there in high concentrations."

The cell wall of most bacteria, including *V. cholerae*, consists of a complex of molecules called peptidoglycans and is separated from the watery cytoplasm of the cell by an inner membrane. Scientists have puzzled over how the inside of the cell coordinates growth with the cell. Waldor's group found that D-amino acids are chemical signals that can downregulate cell wall metabolism when bacteria slow their growth.

His lab group pinpointed an enzyme, called a racemase, that *V. cholerae* uses to create certain D-amino acids and mutated it to test the effects. Without D-amino acids, the cell wall's growth was uncoordinated with the cell's inner growth. The team's findings were published in *Science* on September 18, 2009. ■

–SARAH C.P. WILLIAMS

IN BRIEF

when studying Hox proteins, DNA-binding proteins that help control an organism's development. Different Hox proteins must bind their various DNA targets with high specificity, and scientists didn't know how they did it.

Collaborating with an x-ray crystallographer and a developmental biologist, Honig's lab group previously analyzed images of two different DNA sequences bound to a Hox protein. One sequence was highly specific for that Hox protein; the other was able to bind other Hox proteins as well. They found that the more specific sequence had a narrower groove in its double helix than the other sequence, and this groove was targeted by the protein.

The team's newest findings, published in *Nature* on October 29, 2009, explain why: a narrower groove changes the electrostatic potential of the DNA molecule in that area, facilitating binding to certain proteins. Honig and his collaborators also scanned a database of other DNA-binding proteins to see which would bind to DNA with narrower grooves. They were able to establish some general rules on what protein parts tend to be attracted to narrow grooves. They next want to use this information to develop algorithms to predict how proteins use DNA shape to recognize binding sequences.

PREGNANCY PROBLEM'S LATE EFFECTS

Pre-eclampsia, a complication of pregnancy that leads to high blood pressure and impaired kidney function, seems to resolve itself as soon as the baby is delivered. But scientists have begun to link pre-eclampsia with health problems later in life, including high cholesterol, high blood pressure, heart disease, and stroke. Now, research by HHMI investigator S. Ananth Karumanchi, of Beth Israel Deaconess Medical Center, has also linked it to thyroid problems down the road.

Excess levels of a protein called sFLT-1 lead to pre-eclampsia by blocking VEGF, a protein needed for blood vessel growth and repair, according to Karumanchi's earlier studies. VEGF-blocking drugs are sometimes used to stop the growth of cancerous tumors but these drugs may lead to pre-eclampsia-like signs and symptoms. He noticed that some cancer patients taking VEGF-blocking drugs developed low thyroid function and wondered whether pre-eclampsia patients also had this risk.

Karumanchi combed through data from previous studies and found that women with a history of pre-eclampsia during pregnancy had a one in five risk of low thyroid function over the 20 years following a pregnancy—the normal rate is one in 15 women. The analysis, published online November 17, 2009, in the *British Medical Journal*, could encourage doctors to keep a closer eye on women's thyroid function tests after pre-eclampsia.

GENETIC DIAGNOSIS

For the first time, scientists have diagnosed a genetic disease by sequencing all of a patient's protein-encoding genes—not testing for just one suspected disease. A team led by HHMI investigator Richard P. Lifton used a novel technique to make the rapid diagnosis on an infant in Turkey who was constantly dehydrated and failing to gain weight.

Lifton's lab group, at Yale School of Medicine, started with a blood sample from the patient. Instead of searching the entire genome for a disease-causing mutation, they used a microarray chip that separated out only the protein-encoding DNA—about 1 percent of the genome. Mutations there can lead to proteins being