DNA Repeats Tied to Neuromuscular Diseases

Just two years ago geneticists thought they had fingered a unique genetic mistake as the cause of a disease called fragile X syndrome (SN: 6/8/91, p.359). Since then, researchers have traced three more disorders, including myotonic dystrophy (SN: 2/15/92, p.102) and Huntington's disease, to similar mistakes. Now, the discovery that this kind of mistake – unusual repetition of a short stretch of DNA – leads to yet another neuromuscular disorder has driven home the idea that such errors may account for many genetic disorders of the nervous system.

After decades of searching, two geneticists and their colleagues have demonstrated that chromosome 6 in people with spinocerebellar ataxia 1 contains a short piece of DNA that repeats 40 to 80 times, two to four times more than it should. These excess repeats lead to a progressive destruction of part of the brain, which causes a loss of coordination and, eventually, loss of the ability to breathe or swallow, says Huda Y. Zoghbi, a pediatric neurologist at Baylor College of Medicine in Houston. She, Harry T. Orr of the University of Minnesota in Minneapolis, and their colleagues describe their findings in the July NATURE GENETICS.

Four chemical entities called nucleotides make up the genetic alphabet, spelling out the words and sentences – known as genes – that specify the amino acid sequence of each protein. In some mutations, the loss or substitution of nucleotides in a gene creates words that make no sense, which results in an inactive protein or none at all getting made. But in this ataxia and the other four genetic disorders, the mistake arises when a series of three nucleotides repeats many times more than usual. That error can spell out a new "word" that leads to the creation of an atypical, potentially harmful protein, Zoghbi says.

"I'm sure there are going to be other diseases found to have this mechanism," predicts human geneticist Stephen T. Warren of the Howard Hughes Medical Institute at Emory University in Atlanta.

Orr and Zoghbi found the repeats by examining pieces of the million-base-pair section of chromosome 6 known to be defective in people with this ataxia. They made enough kinds of short, 20-nucleotide fragments to cover all possible repeating threesome combinations. Then they evaluated each repeating section of DNA to see which section varied in the number of repeats in people with the disease. The threesome that repeats involves the nucleotides cytosine, adenine, and guanine.

The number of times this threesome repeats varies from one generation to the next, Orr and Zoghbi say. They observed that older family members with less severe disease had about 40 repeats, but the gene in some of the children contained 80 copies of the three nucleotides. These children developed symptoms much earlier in life. "The bigger the expansion, the more severe the disease," Zoghbi says.

This variability could explain why many so-called genetic diseases fail to appear to the same degree in every generation or family member, says Warren. Previously, researchers thought several genes interacted to cause these baffling disorders, making the identification of their genetic bases too daunting. "Now people are reexamining these disorders," he adds. "And there are a slew of diseases like that."

For example, two other reports in the July NATURE GENETICS pinpoint the DNA responsible for two other types of ataxia. In one paper, Japanese researchers tracked the faulty gene leading to Machado-Joseph disease, which causes nerve and muscle degeneration, to chromosome 14.

In the other, collaborators in England, Cuba, France, Germany, and the United States studied 450 Cubans and seven French families. In those people, spinocerebellar ataxia 2 arises from aberrations on chromosome 12, says Sue Chamberlain of St. Mary's Hospital in London, one of the researchers. Their data suggest that a third type of this ataxia may also exist.

Data from families with these ataxia disorders indicate that one generation suffers mild symptoms as adults while the next generation develops more severe disease, often during childhood. "We would suspect very strongly that those mutations will be unstable trinucleotide repeats," says Orr.

"This commonality [of abnormal repeats] is very exciting," comments Giovanna M. Spinella, a clinical neurologist at the National Institute of Neurological Disorders and Stroke in Bethesda, Md.

Still, many questions remain. The genome contains many sequences of DNA in which two, three, or four nucleotides repeat a half dozen times or more. Scientists do not know why the number of repeats suddenly becomes variable or why excess threesomes, but not twosomes or foursomes, cause problems, especially for the nervous system.

"We have a lot to do now to try and link the pathology [of these diseases] with these repeats," Spinella says. – *E. Pennisi*

Ballast-water invaders pose ecological risk

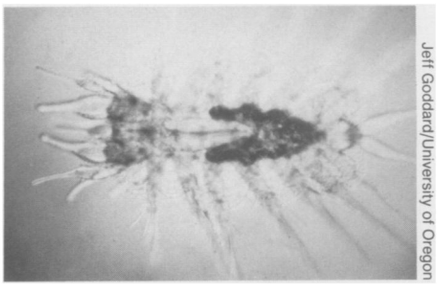
They came, they multiplied, they conquered: In the mid-1980s, zebra mussels hitchhiked to the Great Lakes in the ballast tanks of a transoceanic cargo ship, triggering one of the most disastrous ecological invasions in recent U.S. history. But other ballast-water invaders are reaching saltwater ports, inland waterways, and marine estuaries on a vast and largely unnoticed scale, says marine ecologist James T. Carlton of Williams College in Williamstown, Mass.

Carlton and colleague Jonathan B. Geller of the University of North Carolina at Wilmington counted and identified the creatures residing in the ballast water of 159 ships in Coos Bay, Ore., one of the largest exporting ports in the Pacific Northwest. Water from the ships, which hailed from 25 Japanese ports, contained 367 different marine species, including shrimps, sea anemones, jellyfish, snails, clams, fish, flatworms, and a variety of microscopic life forms, Carlton and Geller report in the July 2 SCIENCE.

"The total diversity was a surprise," Carlton recalls. "We didn't expect to find things like hermit crabs, starfish, or sea squirts."

Since the 1880s, empty and near-empty ships have taken on water as ballast to increase their stability and balance on the open seas. After reaching their destinations and loading cargo, freighters pump the water back out – along with any marine life sucked up into the tanks at the home port.

Dumping ballast water into foreign ports could have dire consequences for native marine creatures and for the people whose livelihoods depend upon them, says Carlton. "All you have to do is insert one new species into a system and the ecological roulette [wheel] is set in mo-



Jeff Goddard/University of Oregon

This scale-worm larva traveled to Coos Bay, Ore., in the ballast water of a Japanese freighter. Such nonindigenous invaders pose ecological hazards to bays, estuaries, and inland waterways on a global scale, researchers say.

tion," he says. But it's very hard to know whether such an introduction will upset the natural balance or prove benign, adds Carlton.

Carlton and Geller emphasize that a significant number of foreign invaders may have established themselves already in U.S. coastal waters. Some will go unnoticed until, like the zebra mussel, they present a major nuisance. Other invaders have been misidentified as native species. "We think the number of invasions is vastly underreported," says Carlton.

Recent events in the Black Sea illustrate the potential hazards of ballast-water dumping. In the early 1980s, the North American comb jellyfish rode a freighter into the Azov Sea, a semi-enclosed body of water in the northern Black Sea. The disruption that followed has virtually wiped out the Azov Sea's anchovy fisheries, causing a "major economic and social disaster," says Carlton.

Closer to home, San Francisco Bay has seen some notable invasions recently. For example, the Asian clam appeared there in 1986, almost certainly transported in the ballast tanks of a freighter, says Peter B. Moyle, a fish biologist at the University of California, Davis.

Today, the bay bottom is covered by 10,000 or more of these creatures per square meter. Moyle fears the clams will out-compete native species for food, retarding the recovery of the bay's declining estuary. And just last year, the European green crab found its way to the bay. Nobody is sure whether this voracious predator will help control the Asian clam invasion or damage the local shellfishing industry, according to Moyle.

"It's a lottery," he says. "Every time one of these ships comes over and dumps water into the system, you never know what's going to make it."

One very important question remains, says marine biologist John W. Chapman of Oregon State University's Hatfield Marine Science Center in Newport: How often do these inadvertently transplanted species actually gain a toehold in foreign harbors and estuaries? "We can speculate," says Chapman, "but there are no data."

– D. Pendick

Exploring gravity, tides, and excited atoms

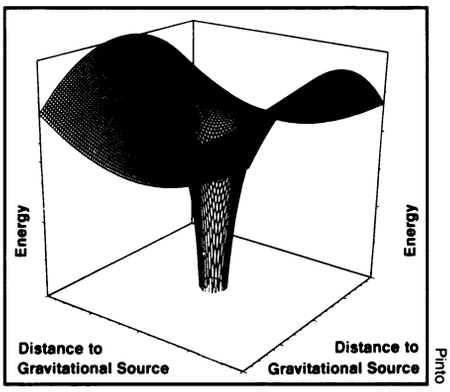
It's hard to imagine how the force of gravity – normally associated with baseballs, planets, and galaxies – could possibly have a perceptible effect on the motion of electrons in an atom, where quantum mechanics and electrical forces reign. But given a sufficiently strong gravitational field and an excited hydrogen atom in which the electron spends most of its time at great distances from the atomic nucleus, such an interaction becomes possible.

A physicist has now established that, in principle, the gravitational field of a compact, dense object such as a neutron star is strong enough to influence loosely bound electrons in hydrogen atoms close to it. Fabrizio Pinto of Boise (Idaho) State University reports his calculations in the June 21 PHYSICAL REVIEW LETTERS.

The idea of studying how a gravitational field may influence the motion of electrons in an atom and, hence, subtly change the characteristic wavelengths of light the atom may absorb or emit goes back more than a decade to the work of Leonard E. Parker of the University of Wisconsin-Milwaukee. He was interested in the possibility of using atomic spectra to measure strong gravitational fields.

Parker's calculations showed that for electrons tightly bound to atoms, only exotic black holes smaller than dust specks had sufficiently strong fields to influence electron energy. However, the situation looked a little more promising for excited atoms with loosely bound electrons.

Pinto carried this research further. His results reveal that electrons in freely falling, excited atoms close to the surface of a neutron star would experience gravitationally induced changes in energy large enough for a radiotelescope to detect.



Pinto

This drawing shows how the strong gravitational field of a typical neutron star affects the potential energy of an electron bound to an atom situated at the star's surface. The star's gravitational force stretches the atom in much the same way the moon induces tides on Earth. This stretching adds a waviness to the corresponding potential energy diagram.

Unfortunately, these excited atoms are also extremely fragile. Detection of a gravitational effect appears possible only at low temperatures and in the absence of significant magnetic fields. This rules out an environment such as the surface of a neutron star, which typically has a strong magnetic field and a high surface temperature. Pinto is now working to identify alternative situations where the gravitational effect may actually be observable.

– I. Peterson

A DNA structure that tags genetic junk?

Although DNA holds the instructions for making proteins, a sizable fraction of these sacred codes appears to contain nonsense. Indeed, after an RNA copy of DNA is made, an elaborate splicing system removes the genetic junk, sequences called introns. The substantive information in the remaining codes, called exons, can be patched together and translated into an enzyme or other useful protein. So why do introns exist at all?

A report in the June 22 BIOCHEMISTRY may provide a clue. Using a chemical probe, two chemists at the California Institute of Technology in Pasadena have discovered a structural landmark that appears to flag introns.

"Here we have what I think is a first indication that introns are structurally delineated at the DNA level," says Jacqueline K. Barton, one of the Caltech researchers.

A regularly occurring structure on DNA should play some important role, she says. Thus, the finding contradicts the view that introns are evolutionary relics that are removed because they serve no purpose.

The work supports the exon shuffling theory, which contends that introns act as spacers where breaks for genetic recombination occur. Under this scenario, exons – which usually contain instructions for building a protein subunit – remain intact when shuffled during recombination. In this way, proteins with new functional repertoires can evolve.

Barton and co-worker Inho Lee were searching viral DNA for unusual structures, which often prove to be biologically interesting. They used a chemical probe containing rhodium, which Barton describes as a "funny-looking metal complex which recognizes funny-looking structures."

The probe, it turns out, bound to specific structures on DNA introns. The researchers shone a light on the DNA to trigger a break at the sites of each of these structures and then determined their locations by studying the cleavage patterns. The structures appeared to occur near the ends of introns, says Barton. The same results emerged for each of the two different viral DNAs they studied.

The new structures may be signposts marking where an intron ends and an exon begins, Barton says. The two chemists are planning additional studies that will compare the cleavage patterns of DNA introns and their RNA analogs. They hope eventually to look for hints of how these structures function. By fishing out which cellular or nuclear components bind to the structures, Barton says, they may be able to deduce the structures' physiological role.

– K.F. Schmidt