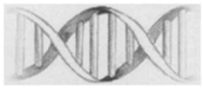
# When CAG Spells Trouble

## DNA repeats may turn good proteins bad

By JOHN TRAVIS



*When Huda Y. Zoghbi, a pediatric neurologist at Baylor College of Medicine in Houston, spoke to a local family marked for seven generations by the inherited disease spinocerebellar ataxia type 1 (SCA1), no one recalled having heard anything dramatic about the long-dead man to whom she had traced the first obvious sign of the illness. He had lived well past his 80th birthday and "just tumbled around a bit late in life. Nobody thought much of it," current family members told Zoghbi.*

By the beginning of this decade, geneticists thought they had a firm handle on most of the ways DNA could get nasty. Then in 1991 came an unexpected report about the genetic flaw underlying fragile X syndrome. The most common inherited form of mental retardation, fragile X afflicts 1 in every 2,500 people, most of them men.

On the X chromosome of affected individuals, a small stretch of DNA had seemingly taken on a life of its own and copied itself over and over. As researchers traced this unstable DNA through family trees, they found that the repeating portion was often larger in each new generation.

Suddenly, the puzzling but well-documented trait of "anticipation," in which a disease strikes earlier and more severely in successive generations, had a possible explanation. Researchers quickly established that other conditions exhibiting anticipation, such as myotonic dystrophy and Huntington's disease, also owed their unusual inheritance pattern to expanding DNA.

More than a half dozen genetic illnesses have now been pinned on this phenomenon, which still goes by various names: unstable DNA, dynamic mutations, triplet repeats. "This is a whole new area of medical genetics. We didn't even know about [these repeats] a few years ago," says Marian DiFiglia of Boston's Massachusetts General Hospital (MGH).

Once past the initial shock of discovering a new mechanism behind inherited diseases, researchers began delving into how repeating DNA wreaks its havoc. Recent work provides some insight into three deadly inherited neurodegenerative illnesses—Huntington's disease, SCA1, and dentatorubral and pallidoluysian atrophy (DRPLA)—caused by these genetic stutters.

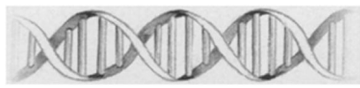
Each of the diseases initially destroys only a specific group of brain cells. SCA1 targets cerebellar neurons called Purkinje cells, while DRPLA devastates different neurons in the cerebellum and other regions of the brain. Huntington's disease ravages so-called spiny neurons in the striatum.

All three are also CAG-repeat diseases, a description that refers to the strip of DNA that proliferates. The building blocks of DNA are four versions of a complex organic molecule called a nucleotide. Designating these nucleotides as C, A, G, and T, geneticists spell out the sequence of any DNA strand using just these letters.

When researchers identified the genes responsible for the three diseases, they found that in unaffected individuals, each cell's two copies of the relevant gene had stretches of DNA where CAG repeated anywhere from half a dozen to nearly 40 times. But in affected people, the CAG triplet occurred from 40 to more than 100 times on one or both copies of the gene.

How does this genetic profusion lead to a disease? In fragile X syndrome (where the repeated nucleotide triplet is CCG rather than CAG), the position of the extra DNA within a gene appears to work by squelching the manufacture of a protein at the transcriptional level. Transcription is the process in which the DNA sequence of a gene is converted into messenger RNA, a molecule that the cell then uses to construct the gene's protein.

If the number of CCG repeats exceeds a still poorly understood threshold, cells produce less and less messenger RNA from the fragile X gene and correspondingly less protein. Geneticists therefore attribute the syndrome to a loss of function, since it appears to result from the absence of a vital protein.



*Three generations after that first family member was affected, some members were bedridden by their sixties and dead by their seventies. SCA1 first destroys neurons in the cerebellum, an area of the brain that helps control balance and coordination. "It eventually kills patients because they can't swallow or breathe," says Zoghbi.*

Not all DNA-repeat diseases work this way. Researchers have shown that in a fourth CAG-repeat illness, Kennedy's disease, the expanded gene pumps out flawed versions of its normal protein, a receptor for the hormone androgen. Since each nucleotide triplet determines a specific amino acid for a protein, the extra CAGs appear to add a long stretch of one amino acid, glutamine, to the receptor's normal sequence.

Groups from France, Japan, and the United States report in the May NATURE GENETICS that Huntington's disease, DRPLA, and SCA1 follow in the footsteps of Kennedy's disease, not fragile X. The CAG repeats on the disease genes apparently translate into extra glutamines, creating larger than normal proteins.

Research teams led by Ichiro Kanazawa of the University of Tokyo and Masao Yamada of the National Children's Medical Research Center in Tokyo collaborated on the DRPLA research. From the sequence of the normal DRPLA gene, they predicted the form of small bits, or peptides, of the resulting protein and synthesized them. They then inoculated rabbits with the peptides, producing antibodies that bind to those fragments.

Because antibodies latch onto proteins containing the peptide that elicited them, researchers use antibodies to probe tissues and portions of cells for the presence and distribution of proteins from the genes in which they're interested. Applying their antibodies to samples taken from both normal and DRPLA-affected human brains, the Japanese groups discovered two proteins. One appeared only in DRPLA

brains, while a smaller one was present in both normal and affected brains. "In short, we have identified normal and mutated DRPLA gene products," they conclude. Furthermore, their antibody tests suggest that both proteins are located outside cell nuclei.

Following a similar strategy, a team headed by Zoghbi and Harry T. Orr of the University of Minnesota probed cultured cells and tissues of normal and SCA1 individuals with antibodies to ataxin-1, the protein made from the normal version of the SCA1 gene. In brain samples from both groups, the antibodies picked up the presence of ataxin-1. In those with SCA1, they also latched onto a larger protein that researchers say is the mutant molecule.

Furthermore, the antibodies provided a potentially significant clue to why the disease attacks specific neurons. In almost all types of brain cells, researchers located ataxin-1 only inside the nucleus. But in the Purkinje cells, they also found it outside the nucleus, in the cell's cytoplasm. "That the one cell that degenerates in the disease has dual signals is interesting," says Zoghbi.

The same antibody techniques lend themselves to investigations of Huntington's disease, a much more common disorder than SCA1 or DRPLA. Jean-Louis Mandel of France's National Center of Scientific Research and his group detail their work on the disease in NATURE GENETICS. Their research mirrors recently published reports from groups headed by Christopher A. Ross of Johns Hopkins University in Baltimore, MGH's James F. Gusella, Richard M. Myers of Stanford University, and Neil Aronin of the University of Massachusetts Medical Center in Worcester. "In general, all our results are pretty comparable," says Ross, whose analysis appears in the May NEURON.

As in the DRPLA and SCA1 studies, antibodies made by these researchers pick out two proteins, one larger than the other, in cells that possess a normal version of the Huntington's gene and a copy marred by too many CAG repeats. But unlike the SCA1 results, staining cells and tissues with the antibodies does not uncover the two proteins in nuclei. The tests instead reveal that both proteins are found throughout the cell body of neurons.

Researchers have not reached a consensus on whether the proteins localize in particular regions of neurons. The groups led by Ross and Mandel found more intense staining in the terminals of nerve cells, hinting that the normal protein may play a role in transmitting messages between neurons. But other researchers say that their antibody assays haven't corroborated that picture. "I don't think we've had enough time to study the whole picture yet," says DiFiglia.

*In the next generation of the family, some of whom Zoghbi examined personally, those with SCA1 began to show symptoms in their forties, had to use a wheelchair by their fifties, and rarely survived much beyond 60.*

Researchers believe that the CAG repeats involved in these neurodegenerative diseases provide a gain of function, in contrast to the apparent loss of function in fragile X. This means that the diseases result not from an absence or scarcity of normal proteins, but from a novel and damaging role instigated by the mutant proteins with their extra string of glutamines. "It does something that it didn't do before," says Myers.

But what are those new functions? Max Perutz and his colleagues at the Medical Research Council in Cambridge, England, last year championed an idea they call "polar zippers," in which the mutant protein's long stretch of glutamines forms a sheetlike structure held together by hydrogen bonds. The mutant protein might then use this structure to join together two other intracellular proteins, which would in some way result in the death of a neuron.

A year earlier, Howard Green of Harvard Medical School in Boston noted that some enzymes have a propensity for attaching glutamine-rich proteins to certain other molecules. Green therefore suggested that these enzymes would connect the mutant proteins, with their extra glutamines, to molecules with which they would normally have no relationship. But like the speculation of Perutz's group, Green's theory presents no clear path from the proposed interactions to neuronal death.

"There's a lot of hypotheses. None of them are particularly satisfying. The bandwagon everyone is jumping on is that proteins interact with other proteins in cells, and the mutant protein may interact differently," comments Myers.

Any gain-of-function hypothesis must pass a stiff test. "The big challenge now is to explain the selective neuronal death," comments Michael Hayden of the University of British Columbia in Vancouver. That's because only in the SCA1 antibody results have researchers found even the smallest hint of a distinction between the neurons targeted by each disease and those spared. In the various Huntington's and DRPLA studies, researchers have discovered no significant differences in the amount or location of mutant and normal proteins when they look at different types of neurons.

To many in the field, this suggests that the discriminating nature of the diseases derives from unique proteins or cellular processes found in distinct classes of neurons. "It's going to come down to why certain neurons are vulnerable. That will relate to the specific biology of these cells," says DiFiglia.

With the initial antibody studies confirming that mutant proteins are indeed produced, two major items top the agenda for those investigating Huntington's disease, SCA1, and DRPLA.

In one effort, studies of protein binding should reveal what molecules attach themselves to the normal or the mutant protein. Those data might uncover what roles the proteins play within cells. As MGH's Gusella puts it, "in essence, there will be a lot of mucking around to look for a clue."

The other major endeavor is to expand the research focus from cells and tissues to whole organisms. "We all eagerly await the findings of the genetically manipulated mice," says Hayden.

On the Huntington's disease front, for example, several groups have reportedly created so-called knockout mice, animals in which the gene that produces the normal version of the disease-causing protein is turned off. By comparing these manipulated mice to normal ones, researchers hope to determine the function of the missing protein. That may prove difficult in this case, however. Though no group has yet published a description of a knockout strain for Huntington's, several researchers told SCIENCE NEWS that the mice die as embryos. This suggests that the protein is critical to development, they say, yet makes it almost impossible to unearth the protein's role in a mature animal.

In addition to knocking out the normal versions of the genes that cause the CAG-repeat illnesses, researchers would like to create animal models of the diseases. They can do this by inserting a CAG-ridden gene into otherwise healthy mice. For example, the Baylor and Minnesota groups have collaborated to generate a strain of mice that mimics the progression of SCA1. They engineered mice whose Purkinje cells manufacture a version of ataxin-1 with extra glutamines. The neurons degenerate quickly, and the mice die within weeks, says Orr.

As these advances indicate, researchers are now moving beyond a state of bewilderment about the genetics of Huntington's disease, SCA1, and DRPLA toward an understanding of the molecular mechanisms at the heart of these illnesses. That knowledge, researchers hope, may one day prevent tragedies like that of the SCA1 family Zoghbi has followed.

*By the sixth generation, family members were dying in their thirties and forties. Today, in the seventh and current generation, SCA1 strikes before adulthood. Two children, age 9 and 15, have already succumbed, says Zoghbi.* □