NEUROBIOLOGY

# An Intriguing New Lead on Huntington's Disease

The discovery of a new disease gene is always a welcome development, but it is not a sure ticket to understanding the disease. Take Huntington's disease, a neurodegenerative condition that strikes in middle age, causing involuntary movements and dementia. Three years ago, researchers found the gene responsible for Huntington's and identified the disease-causing mutation, which expands the length of a repeated stretch of the amino acid glutamine in the gene's product, a protein called huntingtin. But that discovery didn't explain how the expanded glutamine region causes brain neurons to sicken and die. And so researchers began to search again—this time for proteins that interact with huntingtin and might therefore hold clues to its function. That hunt has now led a research team at Duke University Medical Center to a tantalizing finding.

James Burke, Jeffrey Vance, Warren Strittmatter, Allen Roses, and their colleagues at Duke University Medical Center report in the March issue of *Nature Medicine* that huntingtin and another protein that causes a rarer neurodegenerative disease both interact—via their polyglutamine regions—with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). While GAPDH is not the first protein shown to interact with huntingtin, it is the first whose function is known: It is a key enzyme in glycolysis, the essential metabolic pathway by which cells begin converting the sugar glucose to energy.

Burke notes that GAPDH may have other functions as well, but it's the enzyme's role in glycolysis that suggests the most intriguing possibility: that the mutant proteins in Huntington's and four other less common neurodegenerative diseases stunt energy production by interfering with GAPDH. "Assuming that what they see is correct, it is a very exciting finding, and implicates metabolic stress as a common pathogenic mechanism in all the glutamine repeat diseases," says Christopher Ross, whose team at Johns Hopkins University School of Medicine reported a huntingtin-binding protein called HAP-1 in the 23 November 1995 *Nature*.

The Duke group's finding, if it holds up, may also have clinical implications. "This may be one of the better leads on potential treatments of Huntington's disease," says University of Pennsylvania neurogeneticist Kenneth Fischbeck. "You might be able to compensate for a problem with glycolysis." But he and others caution that the Duke team has not yet shown that binding of the mutant form of huntingtin to GAPDH does in fact impair energy production or harm brain and spinal cord neurons.

What spurred Burke and his colleagues to focus on the glutamine-repeat proteins was the recognition that diseases caused by expansion of glutamine sequences are a clear subclass of the so-called triplet-repeat diseases. There are 10 or so known triplet-repeat diseases, all caused by the expansion of a repeated triplet of nucleotide bases in the DNA. Some of the expansions occur outside the genes' protein coding regions, but in Huntington's and four other neurodegenerative diseases the mutations lengthen stretches of glutamine within the proteins themselves.

**GLUTAMINE-REPEAT DISEASES**

| Disease | Defective Protein | Glutamine Repeats Normal | Glutamine Repeats Mutant | Neuronal Regions Affected |
|---|---|---|---|---|
| Huntington's | Huntingtin | 11 – 34 | 37 – 121 | Basal ganglia, cerebral cortex |
| Spinobulbar muscular atrophy | Androgen receptor | 11 – 33 | 40 – 62 | Spinal cord, brainstem, sensory neurons |
| DRPLA | Atrophin | 7 – 23 | 49 – 75 | Cerebellum, brainstem, basal ganglia, spinal cord, cerebral cortex |
| Spino-cerebellar ataxia, type 1 | Ataxin-1 | 6 – 44 | 40 – 82 | Cerebellum, spinocerebellar system, inferior olive |
| Spino-cerebellar ataxia, type 3 | MJD I | 13 – 40 | 68 – 79 | Multiple motor control regions of brain and spinal cord |

"The bottom line is that the [mutation] is the same in all these diseases," says Roses, of the Duke team. And that, he says, suggests that the diseases may all have the same mechanism. Fischbeck, whose team found the first glutamine-repeat mutation, in the gene for a neurodegenerative disorder called Kennedy's disease, adds that a common mechanism would also fit with other data on the diseases: "They have the same age of onset and rate of progression [and] the same kind of pathology, degeneration of different parts of the nervous system."

Of the five affected proteins, the function of only one is known: Kennedy's disease is caused by a mutated receptor for androgen hormones. But Fischbeck says that information hasn't led to an understanding of how the mutation causes disease.

To try to answer that question for all the glutamine-repeat diseases, Burke and his colleagues went fishing for proteins from human brains that bind to stretches of glutamine, and pulled out GAPDH. To see whether GAPDH binds not just to the polyglutamine they had used to fish it out, but to actual glutamine-repeat–containing proteins, they went angling again, this time with GAPDH as bait. The results were encouraging: Among the proteins that bound to GAPDH were huntingtin and the protein that causes a neurodegenerative disease called dentatorubral-pallidoluysian atrophy (DRPLA).

The discovery of the association with an enzyme involved in energy metabolism raised the researchers' hopes that they were on the right track. Flint Beal and his colleagues at Harvard Medical School had found 3 years ago that monkeys injected with a drug that inhibits energy production developed a Huntington-like disease. That, says Burke, suggested that defective energy metabolism may cause neurodegenerative disease.

Moreover, adds Roses, unlike other tissues, which can get energy from fat, the brain relies almost exclusively on glucose for its energy. Because glucose must be processed by the enzymes of glycolysis for its energy to be harvested, interfering with a key glycolysis enzyme like GAPDH could harm brain neurons by stunting their energy production. Arthur Cooper, who studies neuronal metabolism at Cornell University Medical College in New York, agrees that it is likely that impairing the harvesting of energy from glucose "would in the long term be detrimental to these cells."

That notion led the Duke group to the working hypothesis that the normal forms of the proteins bind to GAPDH with little effect, but the mutant forms bind in a way that inhibits the enzyme. They have not shown this to be true, but Roses says they have preliminary evidence suggesting it may be so: Polyglutamine molecules with more than 60 glutamines seem to inhibit the enzyme's activity in the test tube, while shorter stretches

have no effect. If that result holds up, the next step will be to test whether the inhibition occurs with mutant proteins, and whether it kills neurons.

If such a mechanism does occur, the fact that GAPDH binds to both huntingtin and DRPLA raises the possibility that it could be a common mechanism for all the glutamine-repeat diseases. Huda Zoghbi of Baylor College of Medicine in Houston and her collaborators, Pennsylvania's Fischbeck and Harry Orr of the University of Minnesota, have unpublished findings that bolster that notion. They have found that GAPDH binds at least two other glutamine-repeat proteins.

But even if this hypothesis proves true, other important questions will remain, such as how each disease targets a different set of neurons even though each glutamine-repeat protein is widely expressed in the brain, and GAPDH is in every cell. "There may be other proteins that are cell-specific that interact with these glutamine-containing proteins and enhance the interaction with GAPDH," Zoghbi suggests. The HAP-1 protein discovered by Ross's group might be one such protein. But for researchers trying to get to the bottom of glutamine-repeat diseases, the protein hunt is certainly not over.

**–Marcia Barinaga**

IMMUNOLOGY

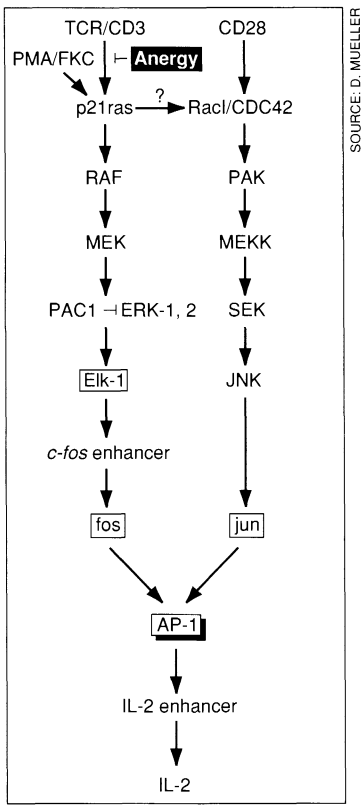
# T Cell Inactivation Linked to Ras Block

Like nuclear missiles, the immune system's key regulatory cells, the T cells, won't go into action against an enemy unless they are triggered by several signals at once. Indeed, cell culture studies show that if a T cell receives only one of the two signals it needs, it becomes paralyzed—unable to divide or produce certain growth factors vital for making immune responses. How this state, known as anergy, is achieved has long puzzled researchers, but two groups, one led by Frank Fitch of the University of Chicago and the other by Daniel Mueller of the University of Minnesota in Minneapolis, report new results in this issue that may help solve the puzzle (see pp. 1272 and 1276).

Both teams' results imply that the cause of anergy lies in a block early in the Ras signaling pathway, one of the pathways most widely used to control growth and differentiation in a diverse range of cells and tissues. "This is strong evidence pointing us toward the block," says immunologist Art Weiss of the University of California, San Francisco. The finding is not only shedding light on the biochemical underpinnings of anergy, but by doing so may help resolve a long-standing debate among immunologists.

Although many believe that anergy may help prevent the immune system from mounting an attack on normal body tissues by putting self-reactive T cells out of action, others have suspected that it's just a laboratory artifact. By pinpointing a specific alteration in one of the cell's major control pathways as the cause of anergy, the new work bolsters the view that it does play an important physiological role. "I don't think anergic cells are playing dead. It looks as if they're doing things with a real function in the immune response," says Mueller. And beyond its implications for immunology, the work will also help understand operation of the Ras pathway itself.

The two groups came to their conclusions by different routes. Mueller's wanted to pin down how the production of a key immune system growth factor, interleukin-2 (IL-2), is blocked in anergic cells. The team took its starting cues from previous work by Michael Lenardo's team at the National Institute of Allergy and Infectious Diseases indicating that the shutdown in IL-2 production is due to the failure of the transcription factor called AP-1 to turn on the IL-2 gene, as it normally does when a T cell is activated (*Science*, 21 August 1992, p. 1134).




SOURCE: D. MUELLER

**Locked up?** A Ras block may inhibit both paths leading to IL-2 production.

Either of two signaling pathways could be responsible for this failure, because anergy ensues when a T cell that has received one signal from an antigen fails to receive a "costimulatory" signal from the so-called B-7 molecules on specialized antigen-presenting cells. To try to find out where the failure originates, Mueller says, "we decided to start in the nucleus [where AP-1 acts] and work outwards toward the membrane" along the two signaling pathways. The strategy paid off when the Mueller group showed that in fact inhibition of the activity of enzymes in both pathways appears to be contributing to the decreased IL-2 production characteristic of anergy.

Meanwhile, Fitch's group had picked up on a blockage in the pathway starting with the T cell's antigen receptor. Previous work from their laboratory had shown that two proteins are markedly less active in anergic T cells than in activated cells. "We suspected these might be the mitogen-activated protein kinases ERK-1 and ERK-2," says Fitch, referring to two enzymes that help transmit signals through the T cell receptor pathway. Their latest experiments confirmed that hunch, and with the ERK pathway now clearly shown to be involved in anergy, the results suggested a key role for one of its most prominent members: the Ras protein, which is the product of the cancer-causing *ras* oncogene and normally passes signals from the T cell receptor to other components in the ERK pathway.

Fitch's group looked directly at Ras activity in both normal and anergic cells that they stimulated with antibodies. They found that while this stimulation produced a marked increase in activated Ras in normal T cells, there was no accumulation of the active form of Ras in anergic cells. This indicates an early block in the pathway, Fitch says. Confirming that, both teams treated anergic cells with a chemical called phorbol myristate acetate (PMA), which activates Ras directly. The result: PMA restores normal function, showing that downstream of Ras the pathway could still operate. Finding a block in Ras activation also makes sense in view of the Mueller group's finding that the activity of the costimulatory pathway, whose signals are picked up by the CD28 protein on T cells, is also reduced in anergic cells. Ras "cross-talks" with that pathway, and so loss of its function in one could also inhibit the other.

To complete the anergy picture, researchers will now want to find just what blocks Ras activation in anergic cells. There are plenty of candidates: Ras is known to have several regulators, and previous studies have also found changes in a number of other molecules in the early steps toward anergy. And there's good reason to push on with the search, says immunologist Ron Germain, at the National Institutes of Health, as the payoff is likely to be a better understanding of both anergy and operation of the Ras pathway generally. "Whatever the real role of anergic T cells," he says, "they are going to tell us something important about signaling."

**–Nigel Williams**