GFP sheds light on protein folding

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SUMMARY

The gene behind a rare neurological disease leads researchers to a "molecular Drano" that may underlie Parkinson's disease and other protein-folding disorders.

Often in genetics research, exploration of an exceedingly rare condition ultimately helps people who are suffering from more common illnesses. This may be the case with torsion dystonia, a disorder caused by an abnormal protein that, when functional, may control how other proteins fold — particularly, alpha-synuclein, one of the culprits involved in Parkinson's disease.

With the help of green fluorescent protein (GFP) from jellyfish and the transparent roundworm *C. elegans*, Guy A. Caldwell and his team from the University of Alabama in Tuscaloosa have discovered the role of an eclectic protein, torsinA. "We have adopted a GFP-based system to show the function of a disease-related protein that for six years had eluded researchers," said Caldwell, an assistant professor of biological sciences and Howard Hughes Medical Institute faculty member. His report of these initial results was the cover story in the Feb. 1 issue of *Human Molecular Genetics*.

Torsion dystonia

Abnormal torsinA causes the sustained muscle contractions, abnormal posturing and repetitive twisting movements that are characteristic of torsion dystonia. Unlike Parkinson's, Alzheimer's and Huntington's diseases, torsion dystonia does not cause neurodegeneration, and only about one-third of the people who inherit the dominant mutation develop symptoms.

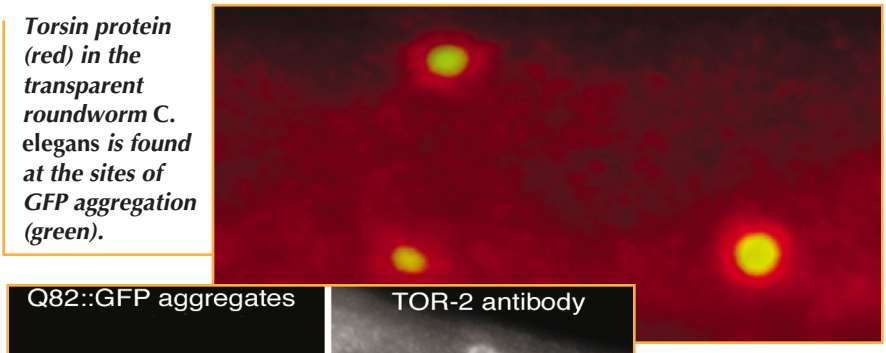
The disorder affects 50,000 people in the general population of North America, but one in 2000 Jewish people of Eastern European descent, a group known as the Ashkenazi. The fact that affected Jewish families today share the same mutation suggests that the problem arose in one individual, and fairly recently.

History explains how this might have happened. In 1648, Ukrainian Cossacks under the leadership of Bogdan Chmielnicki massacred one-quarter of the Polish population, shrinking the Jewish community to a few thousand. From 1800 on, conditions improved and survival increased, mostly among large, wealthy Jewish families. The initial small surviving group included at least one individual with the torsion dystonia gene, which became amplified in the growing population.

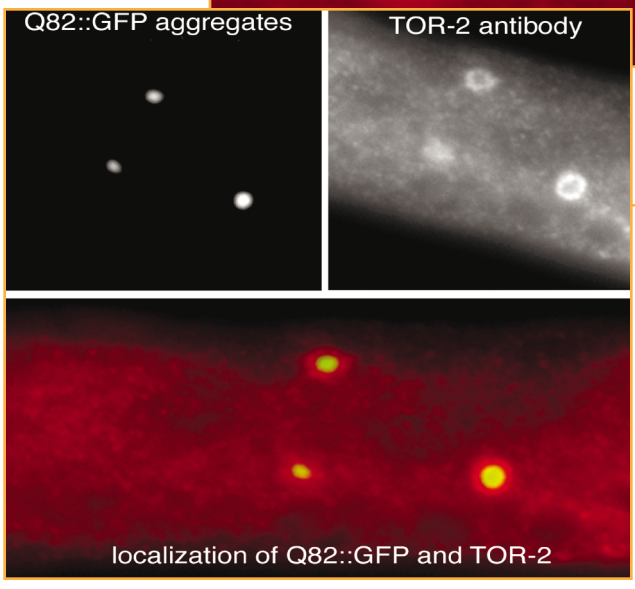
The modern chapter of the story started in 1997, when Caldwell read a report by lead author Laurie Ozelius, now at Albert Einstein College of Medicine in New York, that identified the torsion dystonia mutation in Jewish families as missing three DNA bases near one end of the gene. What intrigued Caldwell was what the paper *didn't* say — that is, what torsin does.

"I looked at the protein and thought it could be something interesting and decided that maybe the worm could be a way to study this," he recalled. He also knew that the implications could transcend this rare genetic disease because Dr. C. Warren Olanow, chairman and professor of neurology at Mount Sinai School of Medicine in New York, had found that torsinA aggregates with Lewy bodies, the brain lesions associated with Parkinson's disease. Ozelius and colleagues found torsinA binding to alpha-synuclein, the principal protein component of Lewy bodies.

At the time, Caldwell was working in the Columbia University laboratory of Martin Chalfie, who was involved in the discovery of GFP's biological use. Chalfie advised



Torsin protein (red) in the transparent roundworm* C. elegans *is found at the sites of GFP aggregation (green).



A view of the GFP aggregates alone (upper left), torsin alone (upper right) and superimposed in color (bottom) reveals that they colocalize at the sites of aggregation.

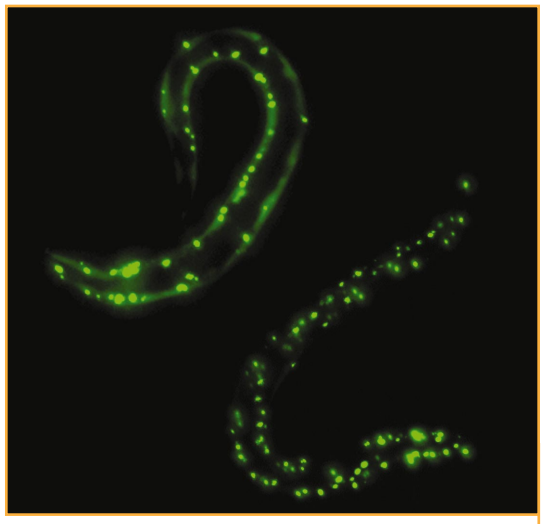
him to wait until he had his own lab before pursuing a jellyfish/worm route to solving the mysteries of human conditions.

Upon arriving at the University of Alabama in 1999, Caldwell wrote a grant proposal to the Dystonia Medical Research Foundation, which, he said, funded his "crazy idea. Without an ounce of data, they believed in my ideas and took a chance on a new young professor."

The worm would provide a good model to probe the relationship between torsinA and alpha-synuclein, he thought, not only because it is perhaps the best-understood animal on the planet, but also because it has a version of the torsinA gene — three of them, in fact. To assess the effects of normal and abnormal torsin expression in the worm, Caldwell's group first expressed, in the same cells, a worm torsin gene along with GFP genes that had been fused to varying numbers of repeats of three bases (C,A,G) that encode the amino acid glutamine. They used these glutamines to test whether proteins folded properly, with or without torsin. (Several disorders of protein misfolding, such as Huntington's disease, are caused by extra glutamines, although Parkinson's disease is not one of them.) Assessing the effect of torsin on protein aggregation would be as simple as viewing the worm's abdomen, thanks to GFP.

Several sets of experiments highlighted torsin's role in protein folding and accumulation. First, the group generated worms that, in specific cells, carried a gene built of GFP linked to either 19 or 82 CAG repeats. In the normal condition of 19 repeats, a smooth, diffuse greenish glow emanated from the muscles of the body wall. But in worms with 82 repeats, the fluorescence appeared as lumps, indicating protein aggregation.

The next experiments generated worms with functional worm torsin genes linked to 19 or 82 CAG repeats marked with GFP (in addition to the worms' normal torsin genes). As expected, worms with 19 repeats with or without extra torsin showed the normal diffuse pattern, but those that contained 82 copies of CAG and that also received a torsin boost had dramatically fewer and smaller fluorescent aggregates.



The animal on the right has fluorescent aggregates that result from an expansion of 82 glutamine residues fused to GFP. In the worm on the left, torsin overexpression partially blocks this aggregation, even though the animal has the same 82 glutamine-GFP fusion protein.

That is, the extra torsin apparently blocked the protein clumping that the 82 repeats would have otherwise induced.

To test this conclusion, Caldwell repeated the experiments using a mutant torsin gene, and the resulting abnormal torsin protein was "completely incapable of restoring the diffuse body-wall fluorescence in these animals," he said. Worms with the human torsin gene had the same results, indicating how greatly this gene has been conserved in evolution.

Sorting it out

The results can be somewhat confusing, Caldwell acknowledged, because they involve several disorders. "Defective torsin causes a movement disorder — dystonia — and torsin is colocalized to sites of protein aggregation in the brains of people who have died of Parkinson's disease. So it *is* convoluted — a protein linked to dystonia may help reduce effects related to Huntington's but is also present in Parkinson's patients!"

Adding to the confusion is that overt protein aggregation is *not* a known part of torsion dystonia. Caldwell theorized that more subtle cellular stresses accumulate from the abnormal torsin, eventually causing the uncontrollable twisting. "Molecules like torsins that may be involved in the mechanism of protein management could influence subtle changes in other proteins that may vary with the extent of environmental or physical stress. The fact that disease progression worsens with time is another reason to hypothesize that cumulative cellular stresses could be involved," he said.

More recent experiments are focusing on Parkinson's disease. "We have shown, in preliminary experiments, that torsin can prevent formation of Parkinson's disease-related protein aggregates," he said.

In January, the Michael J. Fox Foundation for Parkinson's Research funded this group as one of only 11 in the world to investigate torsin further. "This research could lead to new models of the process of neurodegeneration, and we anticipate that the outcomes will have great relevance not only to Parkinson's disease, but to Alzheimer's and amyotrophic lateral sclerosis research as well," said J. William Langston, chief scientific adviser to the foundation.

Although the torsion dystonia mystery that inspired the research has yet to be solved, Caldwell sees other roads to pursue. "Because alpha-synuclein is not a triplet repeat protein, this may indicate that torsins have a more general capacity to prevent protein misfolding. Maybe they can work to suppress the beta-amyloid fibrils that underlie Alzheimer's disease, too. It is our hope that torsins are a form of 'molecular Drano' that work on lots of different clogs."

Caldwell hopes that the work on torsin will come full circle and finally explain how torsion dystonia occurs. And the quest is personal. "In high school I played in a rock band but lost contact with most of the guys. Now, 20 years later, the saxophone player read about my research in a hometown newspaper. Recently, his sister had a child with torsion dystonia. We've reunited after all these years, via dystonia." The friend was tested and, indeed, has the defective gene himself, although he is among the lucky 70 percent who, so far, have not developed symptoms. And he, too, has a young child. "We hope that his daughter will be one of the lucky ones as well," Caldwell said. □