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Researchers Determine Structure of Protein Involved in Spastic Paraplegia

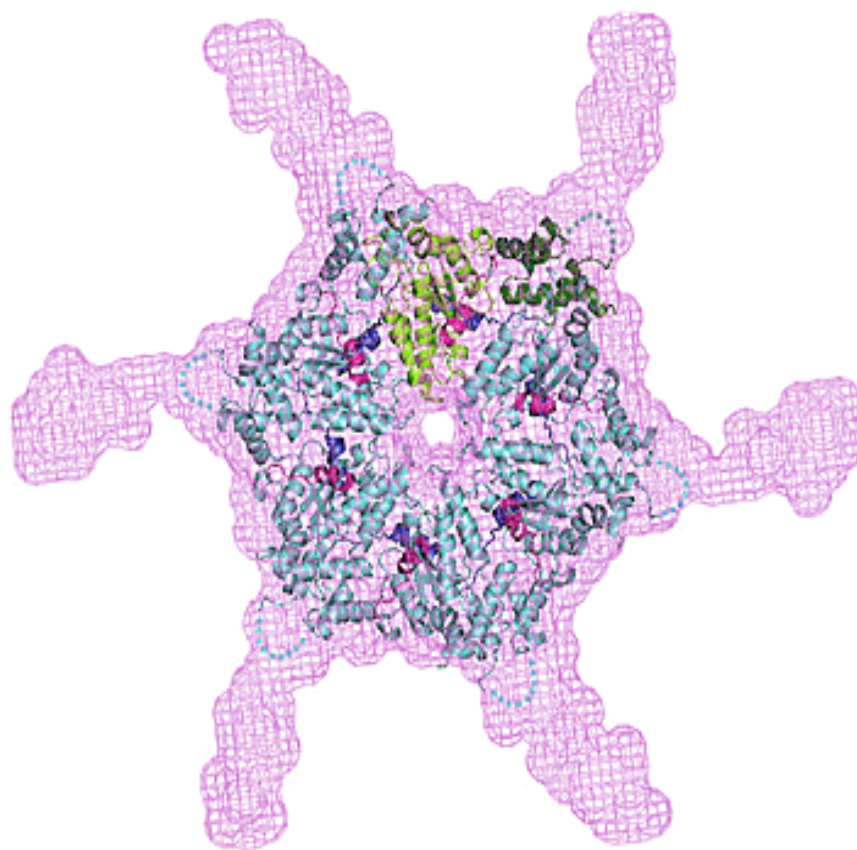


Image Title: A structural model for the spastin hexameric ring derived from small angle x-ray scattering and x-ray crystallography. The radial arms are thought to bind to the microtubule, while the central ring (filled with ribbons reflecting the atomic structure) is proposed to use ATP energy to ratchet a piece of the tubulin protein into the central pore. - Laboratory of Ron Vale, HHMI at UCSF

By piecing together the detailed structure of a molecule-munching enzyme, researchers from the Howard Hughes Medical Institute (HHMI) have revealed how it helps maintains cells' internal highways. The finding could

one day lead to new treatments for a neurological disorder caused when the enzyme, known as spastin, malfunctions.

Spastin functions as a microtubule dismantler, remodeling the network of microscopic conduits that transport molecules around cells. The first atomic resolution structure of spastin, published in the January 17, 2008, issue of the journal *Nature*, suggests that it functions by slurping up a segment of the microtubule protein into a central pore, and then a set of molecular ratchets pulls upon the microtubule until it eventually breaks.

Mutations in spastin have been implicated in hereditary spastic paraplegias (HSPs), a group of disorders characterized by progressive weakness and stiffness in the legs that can lead to complete loss of function. The disease, which affects about 20,000 people in the United States, can also cause mental retardation and abnormalities of the retina and skin. Despite evidence that spastin plays a role in HSPs, however, “the biology of the protein and the cellular pathological of the disease is still very mysterious,” said Ronald Vale, an HHMI investigator at the University of California, San Francisco, who led the study.

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- Ronald D. Vale

One theory, said Vale, is that spastin might be breaking apart microtubules as part of an ongoing process of microtubule network “remodeling.” If microtubule remodeling is inhibited in some of the body's longest nerve cells due to a loss of spastin function, said Vale, the result might eventually lead to spastic paraplegias. But little was known about how spastin does its job, making it difficult to truly understand its role in disease. Vale and his colleague Antonina Roll-Mecak hoped that deciphering its structure might change that.

From previous studies and comparison with similar proteins, Vale and Roll-Mecak suspected that spastin is actually a group of six identical enzyme subunits, linked in a ring, that use energy derived from hydrolyzing the small molecule ATP to break the microtubule. To probe exactly how this occurred, the researchers obtained the protein from the fruit fly *Drosophila* and the worm *C. elegans*, and used two different x-ray techniques to determine the structure of the intact enzyme, as well as a single spastin subunit.

The detailed structures they obtained, as well as functional tests of spastin-mediated severing in a test tube, offer a new view of how spastin

grabs and severs microtubules. “Spastin appears to grab a loose tail region of the microtubule and mechanically ratchet it through the pore, a kind of noodle-slurping mechanism used by other ring-shaped enzymes that are close relatives of spastin. We also find that some microtubules in neurons might be more susceptible to these actions of spastin than others” said Vale.

Beyond this new understanding of how the enzyme functions in healthy cells, Vale and Roll-Mecak's study pinpointed the exact location of disease-causing mutations, thus revealing how changes in spastin's structure might cause HSP. They selectively mutated the ATPase gene in several ways, hoping to glean information about how these altered that enzyme's function. “Some of the mutations we tested were known from human genetics to give disease phenotypes,” Vale said. “Most of those disease-related mutants are very detrimental to the function and activity of the protein.”

Significantly, said Vale, different spastin mutations found in patients with HSP interfere with distinct steps in the microtubule-severing process. For example, he said, some mutations most likely affect spastin's ability to pull microtubules through its core, while others affect binding of the energy-containing molecule ATP. Still others seem to block spastin's ability to form its six-membered ring structure.

“With this structure, we can pinpoint a number of different kinds of ways in which these disease mutations interfere with the function of this protein,” he said. Further work is needed, he noted, to link the findings directly to spastin's role in human disease. “While this structure has given us important insights into spastin's structure and mechanism, we have just scratched the surface in understanding this protein,” he said. “We still need to understand whether spastin is performing the same kind of reaction in the living neuron that we see in the test tube,” he said.